UNIVERSITY
OF MICHIGAN
MAY 17 1955
SCIENCE
LIBRARY

VOLUME 45 MAY, 1955 NUMBER 5

Phytopathology

An International Journal

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

CONTENTS

Thomas Franklin Manns, 1876-1954 — J. W. Heuberger 241

Symposium on Cooperative Agricultural Research in the Western Hemisphere:

Wheat rust races in South America — José Vallega 242

Species of Solanum (Tuberarium) of South America. Present taxonomic status and species used in plant breeding with special reference to Peru — C. Ochoa 247

Late blight resistance—present status — L. E. Heidrick 250

Control of damping-off of sugar beet by Bacillus subtilis — John Dunleavy 252

The role of a self-inhibitor in the germination of rust uredospores — Paul J. Allen 259

Pathological anatomy of bacterial canker of young tomato plants — T. S. Pine, R. G. Grogan, and Wm. B. Hewitt 267

A mosaic disease of Himalaya blackberry — Stanley M. Alcorn, Stephen Wilhelm, and H. Earl Thomas 272

Adult plant resistance to crown rust of certain oat selections — M. D. Simons 275

Disease cycle of Septoria disease of oats — M. D. Huffman 278

Activity of an antiviral agent from Nocardia on two viruses in intact plants — Reed A. Gray 281

Time of peach twig infection by Xanthomonas pruni in relation to spring-canker incidence — Angelos V. Adam, Dwight Powell, and H. W. Anderson 285

Phytopathological Notes:

Antagonistic action of Helminthosporium sativum and Gibberella zeae to infection by Tilletia spp. causing bunt of wheat — Harry W. Schroeder 288

Calcium deficiency and infection of Nicotiana glutinosa by tobacco mosaic virus — M. Chessin and H. A. Scott 288

White spot of alfalfa as a genetic character — Fred Reuel Jones 289

Phellem as a diagnostic symptom of internal cork of sweet potato — A. S. Williams and L. M. Nielsen 290

The effect of organic acids on tobacco mosaic virus multiplication — Farid Nour-Eldin 291

A crown rot of sweet corn caused by Helminthosporium turcicum — R. S. Cox and E. A. Wolf 291

Infection of peach with apple mosaic virus — Hugh C. Kirkpatrick 292

Asexual reproduction in Cephalosporium gregatum — Richard I. Hamilton and Michael G. Boosalis 293

News

Subscription, $10.00 per year

Office of Publication—Monumental Printing Company, Baltimore, Md.

Entered as second-class matter at the post office at Baltimore, Md.

An Invitation to Expanded Research....

We believe the effects of ORTHOCIDE on improving the storage keeping qualities of fruit merit expanded research during 1955 . . . . .

Continuing test work in 1954 with ORTHOCIDE on fruits—together with reports from commercial growers—point to impressive profit gains for the grower through greatly improved *keeping* qualities. A growing number of grower reports show ORTHOCIDE'S beneficial effects on storage and transit keeping qualities of apples, peaches, cherries and strawberries.

Peaches from New Jersey test plot, shown after 23 days in cold storage. 100% of the untreated peaches had rotted; 47% of the sulfur-sprayed peaches had rotted; but all of the ORTHOCIDE-treated peaches were clean and free of rot. After an additional 8 days at room temperature, all of the sulfur-treated peaches had rotted; but 50% of the ORTHOCIDE-treated crop was still saleable. We feel that ORTHOCIDE'S control of Brown Rot is of real importance, because in transit, in storage, in the retail and the consumers' possession—Brown Rot losses run unbelievably high.

ORTHOCIDE has also demonstrated effective field control of various diseases in tests and commercial trials on other fruits. . . .

Cherries . . . Brown Rot and Leaf Spot

Strawberries . . . Botrytis Rot

Apples . . . Scab, Sooty Blotch and Brooks Fruit Spot

Citrus . . . Brown Rot

Extension, state experiment station and grower reports on these fruits are available to you upon request.

Suggestions in answer to technical questions concerning ORTHOCIDE on storage rots and keeping qualities may be obtained by addressing inquiries originating in the 11 Western states to Dr. Thompson . . . . in the Mid-west and East to Dr. Reed.

DR. T. W. REED
Assistant Mgr., Research & Development
California Spray-Chemical Corp.
Kresson Road
Haddonfield, New Jersey

DR. R. K. THOMPSON
Research Coordinator, West
California Spray-Chemical Corp.
Lucas and ORTHO Way
Richmond, California

It is indeed hoped that you will see fit to consider ORTHOCIDE 50 Wettable in your various research studies, exploring this and other fields that you may consider of merit.

Cordially yours,

LEO R. GARDNER,
Manager Research & Development

ORTHO

TM'S ORTHOCIDE, ORTHO, REG. U.S. PAT. OFF.

PHYTOPATHOLOGY

An International Journal

Official Organ of The American Phytopathological Society

EDITORS

A. F. Ross, Editor-in-Chief, Cornell University, Ithaca, New York

C. S. Holton, State College of Washington, Pullman, Washington

Ralph M. Caldwell, Purdue University, Lafayette, Indiana

S. E. A. McCallan, Boyce Thompson Institute, Yonkers 3, N. Y.

ASSOCIATE EDITORS

Vincent W. Cochrane, Wesleyan University, Middletown, Connecticut

Frank P. McWhorter, Oregon State College, Corvallis, Oregon

Earle W. Hansen, University of Wisconsin, Madison, Wisconsin

Edward O. Olson, Citrus Rootstock Investigations, Box 144, Weslaco, Texas

M. T. Hilborn, Agricultural Experiment Station, Orono, Maine

Mortimer P. Starr, University of California, Davis, California

W. J. Hooker, Michigan State College, East Lansing, Michigan

Albert L. Taylor, Plant Industry Station, Beltsville, Maryland

W. F. Jeffers, College Heights, Hyattsville, Maryland

Arthur Kelman, North Carolina State College, Raleigh, North Carolina

R. P. True, West Virginia University, Morgantown, West Virginia

C. E. Yarwood, University of California, Berkeley, California

BUSINESS MANAGEMENT

Saul Rich, Business Manager, 32nd St. and Elm Ave., Baltimore, Md., or P. O. Drawer 1106, New Haven 4, Connecticut.

L. J. Alexander, Advertising Manager, Agricultural Experiment Station, Wooster, Ohio.

NOTICES

Send communications regarding subscriptions and back numbers to Saul Rich, Business Manager, 32nd St. and Elm Ave., Baltimore, Md., or P. O. Drawer 1106, New Haven 4, Connecticut.

Subscription price. $10.00 per year, domestic and foreign. Single current numbers (within 60 days of publication), $1.00. The journal has been issued monthly beginning with Volume 8. **Separate Numbers** may be purchased from back volumes at $1.00 per copy. Complete unbound volumes, 1 through 7, when available, $6.00 each; 8 through 41, as available, $10.00 each. **Thirty-Year Index** (Vols. 1–30, 1911–1940), bound: $7.50, domestic and foreign. **Ten-Year Index** (Vols. 31–40, 1941–1950), paper cover, $3.50. **Lost Numbers.** Claims for copies (domestic subscriptions) lost in the mails must be received within 60 days from the date of issue (Jan. 15 and monthly thereafter); otherwise the usual charge of $1.00 will be made for replacements. Deliveries overseas are not guaranteed and foreign mailings are made at the subscriber's risk. **Separates.** No gratis copies supplied. Quotation is sent with proof.

Advertisements. Rates may be secured from the Advertising Manager. No indorsement of any statement of claims made in advertisements is assumed by this Journal or by The American Phytopathological Society.

Manuscripts and editorial correspondence should be sent to the Editor-in-Chief. At least 1 of the authors must be a member of the American Phytopathological Society. Type on only 1 side of sheet; double-space all parts of manuscript, including footnotes, citations, and legends. For papers longer than 1 printed page, the article should begin with a brief, concise summary. Number footnotes consecutively with arabic numerals, except in tables where lower case letters are used. Give each table a heading stating its contents. Make references to literature as footnotes only when 5 or fewer citations are given. Name and address of the institution where the work was done should precede "Literature Cited." List references alphabetically in "Literature Cited" and number consecutively throughout text as well as in list of citations. Authors are advised to read "Preparing manuscripts for Phytopathology" by W. C. Price, published in Phytopathology 44: 667-674, 1954, as well as current issues of Phytopathology.

Illustrations must be limited in number and should be sharp and clear. One-half the cost of engraving in excess of $16.00, the approximate cost of a single full page cut or of two 3 in. x 4½ in. cuts, are charged to the author. No charge is made for line drawings that substitute for tables unless drawings and tables together exceed 30 per cent of the length of the article. Illustrations are run as text figures designated by arabic numerals. Units of a composite illustration are designated by letters. Arrange full-page figures to conform to journal proportions (6⅛ x 8½ in.), making due allowance for reductions. Specific reference to each illustration must be made in the text, and each must be accompanied by an adequate legend, typed double-space on a separate paper and not on the illustration.

The responsibility for statements, whether of fact or opinion, rests entirely with the writers thereof.

The American Phytopathological Society

FOUNDED 1908. INCORPORATED 1915.

THE A. P. S. COUNCIL

President—James H. Jensen, Provost, Iowa State College, Ames, Iowa
Vice-President—George W. Fischer, Washington State College, Pullman, Washington
Secretary—Glenn S. Pound, University of Wisconsin, Madison 6, Wisconsin
Treasurer—Saul Rich, Connecticut Agricultural Experiment Station, P. O. Drawer 1106, New Haven 4, Connecticut
Editor-in-Chief—A. F. Ross, Cornell University, Ithaca, New York
President-Elect—Helen Hart, University of Minnesota, St. Paul 1, Minnesota
Retiring President—George F. Weber, University of Florida, Gainesville, Florida

Councilors:

E. C. Blodgett, Irrigation Experiment Station, Prosser, Washington
S. J. P. Chilton, Louisiana State University, Baton Rouge, Louisiana
Paul R. Miller, Plant Disease Survey, U. S. Department of Agriculture, Beltsville, Maryland
E. G. Rex, New Jersey Department of Agriculture, Trenton, New Jersey
J. R. Shay, Purdue University, Lafayette, Indiana
W. C. Snyder, University of California, Berkeley, California
W. J. Zaumeyer, U. S. Department of Agriculture, Beltsville, Maryland

DIVISIONS OF THE SOCIETY

Northeastern: A. B. Burrell, Cornell University, Ithaca, New York, *Pres.;* A. C. Tarjan, University of Rhode Island, Kingston, Rhode Island, *Vice-Pres.;* B. H. Davis, Rutgers University, New Brunswick, New Jersey, *Sec.-Treas.*

North Central: Donald Cation, Michigan State College, East Lansing, Michigan, *Pres.;* C. O. Johnston, Kansas State College, Manhattan, Kansas, *Vice-Pres.;* M. B. Linn, University of Illinois, Urbana, Illinois, *Sec.-Treas.*

Pacific: George A. Zentmyer, Citrus Experiment Station, Riverside, California, *Pres.;* S. M. Dietz, Oregon State College, Corvallis, Oregon, *Vice-Pres.;* Roy A. Young, Oregon State College, Corvallis, Oregon, *Sec.-Treas.*

Potomac: H. Rex Thomas, Plant Industry Station, Beltsville, Maryland, *Pres.;* J. G. Leach. West Virginia University, Morgantown, West Virginia, *Vice-Pres.;* L. O. Weaver, University of Maryland, College Park, Maryland, *Sec.-Treas.*

Southern: T. W. Graham, Pee Dee Experiment Station, Florence, South Carolina, *Pres.;* J. A. Lyle, Alabama Agricultural Experiment Station, Auburn, Alabama, *Vice-Pres.;* G. B. Lucas, Plant Pathology, North Carolina State College, Raleigh, North Carolina, *Sec.-Treas.*

SUSTAINING ASSOCIATES

American Tobacco Company, Inc., Research Laboratory, 400 Petersburg Turnpike, Richmond 24, Virginia.

The Dow Chemical Company, Midland, Michigan

E. I. du Pont de Nemours & Company, Incorporated, Wilmington 98, Delaware

Ferry-Morse Seed Company, P. O. Box 778, Detroit 31, Michigan

Freeport Sulphur Company, New Orleans 5, Louisiana

H. J. Heinz Company, Pittsburgh 30, Pennsylvania

Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

Mathieson Chemical Corporation, Mathieson Building Baltimore, Maryland

Rohm & Haas Company, Washington Square, Philadelphia 5, Pennsylvania

Shell Chemical Corporation, Julius Hyman & Company Division, P. O. Box 2171, Denver 1, Colorado

Tennessee Corporation, 619-27 Grant Building, Atlanta 1, Georgia

Young Brothers, Inc., Rochester, Ohio

THOMAS FRANKLIN MANNS, 1876-1954

J. W. Heuberger

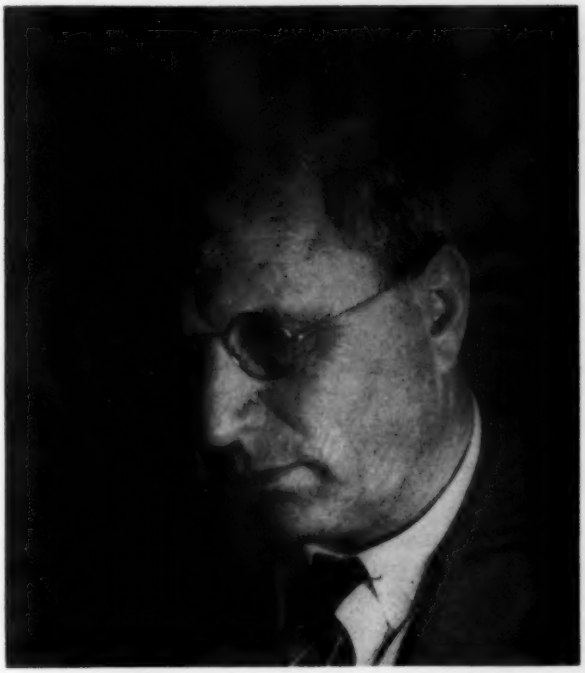
Death came suddenly by coronary attack to Dr. T. F. Manns, Professor Emeritus of Plant Pathology and Soil Bacteriology, University of Delaware, at age 78, during the night of December 22, 1954, at his home in Newark, Delaware. He is survived by two brothers, a sister, his wife, four sons, and seven grandchildren.

Dr. Manns was born in Logan Province, Ontario, Canada, on November 7, 1876; when he was four years old, his parents moved to Jamestown, North Dakota. He spent his youth on a pioneer farm in North Dakota. Later, he attended North Dakota College, graduating with a B.S. degree in 1901 and an M.S. degree in 1903. He obtained his Ph.D. at the University of Pennsylvania in 1913. He was Assistant Botanist, North Dakota State College, 1901-1904; Principal and teacher in the secondary school system in the Philippine Islands, 1904-1907; Assistant Botanist, Ohio Agricultural Experiment Station, 1907-1910, and Soil Bacteriologist, 1910-1912. In 1912 he became Head of the Department of Plant Pathology and Soil Bacteriology, University of Delaware, and remained in that position for 35 years; during the period 1937-1939 he was also Plant Pathologist for the Delaware State Board of Agriculture. Upon his retirement from the University of Delaware in 1947 he became Research Pathologist and Entomologist, Grocery Stores Products Co., Kenneth Square, Pa., working on mushrooms. He retired from that position in 1952 and became a consultant for a mushroom supply house. Then, in 1953, he established his own business producing supplements to increase mushroom production.

His long list of scientific contributions covers many fields, outstanding among them being his work on soil organisms (particularly the actinomyces), nitrogen fixation, copper as a soil amendment, diseases of sweet potatoes, the bacterial spot disease of peach, and the yellows diseases of peach. His most recent bulletins are "Peach Yellows and Little Peach," Delaware Agricultural Experiment Station Bul. 236, 1942; and "Bacterial Spot of Peach and its Behavior in Delaware," Delaware Agricultural Experiment Station Bul. 258, 1945.

He was a charter and life member of the American Phytopathological Society and a charter member and past president (1945) of its Potomac Division. He was a member of the Botanical Society of America; the International Soil Science Society; Sigma Xi; Phi Kappa Phi; the Peninsula Horticultural Society (Secretary, 1937-1947); the Jersey-Delmarva Plant Pathologists Association; Newark Rotary Club; Newark Chamber of Commerce; and the Newark Methodist Church.

Dr. Manns had an inquisitive and inventive mind that ranged far afield from plant research. In 1925, he invented and obtained a patent on an apparatus for determining the specific gravity of tomato pulp. During World War II he submitted many ideas to the Government, among which was one for a submarine anti-detection device, and one for a rocket gun similar to the "bazooka."



THOMAS FRANKLIN MANNS, 1876-1954

In his day, Dr. Manns was a noted athlete. While at North Dakota College he played all sports and won a varsity football letter for 5 consecutive years and captained the team for 3 years (a unique record). His interest in sports continued throughout his life and he played football until age 50 and softball until he was 70 years old.

Perhaps Dr. Manns will be most remembered for his work with youth. He helped establish the Boy Scout movement in Newark in 1912 and was extremely active in youth work in his church. As a teacher at the University of Delaware he stimulated in his students a deep interest in science and life. His home was always open to friends, and he gave of himself freely that others might be happier. Of him it can be said that he practiced the art of giving and enjoyed a full, active life.

PHYTOPATHOLOGY for April (45: 187-240) was issued April 12, 1955

WHEAT RUST RACES IN SOUTH AMERICA [1]

José Vallega[2]

SUMMARY

Although the investigations on the physiologic specialization of cereal rust pathogens in South America have not always been thorough and continuous, they have demonstrated the changes occurring in rust reaction in the main wheat areas of this part of the American hemisphere. They serve also as a good background for further research. The results show that physiologic races as now classified are groups of pathogenical entities which have in common some characters for pathogenicity but which differ in others.

The reaction of the cultivated wheat varieties to rusts and the results from the wheat breeding carried out in the South American countries show almost without exception that each increase in the acreage of a resistant variety is followed by the spread of a race or races able to attack it. Sometimes it is a known but rare race of the pathogen, sometimes it is a new or previously undetected one. This shift in race population is due in part to the fact that the pathogens are able to undergo inumerable and unexpected genetic changes even in the areas where the sexual stages do not occur. This suggests that mutation is more common in the rust fungi than has been recognized previously or that other genetic mechanisms play a role in inducing variability. Basic research on the analysis of the genetic characters for pathogenicity in the pathogens and their relationship to the genetic factors for resistance in the host represents an essential first step in the solution of the problem.

The present paper is a summary of the research in South America on physiologic specialization of the wheat rust pathogens. It includes not only the writer's work, but also the results of others who kindly contributed their unpublished data.[3]

During the first years of the present century, G. Gassner, following Eriksson's concept of specialization, made observations on physiologic specialization of the cereal rusts in Uruguay. In Peru, E. V. Abbot (1) in 1929 identified race 15 of *Puccinia graminis* f. sp. *tritici* and a second one which was able to infect all the differential varieties. Between 1930 and 1932, G. Rudorf and associates (10, 11) made the first identifications of races of *Puccinia glumarum*, *P. graminis*, and *P. triticina* in Argentina. In 1937, W. Straib (15) published the characteristics of the races of *P. glumarum* that he studied in Germany on samples received from Chile, Uruguay, and Argentina. In 1938, the author (17), with the help of Dr. E. C. Stakman and his co-workers, studied at the University of Minnesota the races of *P. graminis* present in Argentina, Chile, and Uruguay. The survey of the races of the cereal rust pathogens was organized in Argentina in 1939 including cooperative work with the neighboring countries. Since 1939, research on physiologic specialization in cereal rusts has expanded greatly in several South American countries (2–6, 8, 9, 11–14, 17–24, 27–29, 30, 31). Studies have been reported by R. A. Da Silva (12–14) and M. Bastos Lagos in Brasil; by D. Volosky de Hernandez (30, 31) in Chile; by C. Boasso and M. N. Levine (2) in Uruguay; by V. A. Revilla (8), E. Rojas Mendoza (9), García Rada (4), Postigo and W. Loegering in Peru; by Coronel in Bolivia; by E. Favret (3, 27–29), H. Cenóz, E. Godoy (5, 6), H. J. Savoia, E. Martinez, O. Bruni, R. H. Batallanez, and the author (16–29) in Argentina. Dr. E. C. Stakman and co-workers of the U. S. Federal Rusts Laboratory and the Minnesota Agricultural Experiment Station studied in detail the races of *P. graminis* f. sp. *tritici* from several South American countries. They also contributed help to everyone concerned with similar problems in Latin America.

PUCCINIA GRAMINIS F. SP. TRITICI.—The surveys made in several South American countries through 1952 have shown the following races present: 48 and 15 in Colombia; 48 in Ecuador; 14, 15, 17, and 189 in Peru; 14, 15, 17, and 11 in Bolivia; 14, 15, 17, and 11 in Chile; 17, 42, 11, and 15 in Brasil, Uruguay, and Argentina. Also, races 178 in Colombia; 9 in Peru; 24, 29, 38, and 48 in Chile; 10, 14, 19, 38, and 56 in Brasil; 21 and 36 in Uruguay have been identified occasionally (Fig. 1).

Collections from several countries were identified as race 29, but it is possible that some of these were race 17 which, under some greenhouse conditions, produces mesothetic reactions on the differential varieties of *Triticum durum*.

[1] Invitational paper presented September 9, 1953, at the Symposium on Cooperative Agricultural Research in the Western Hemisphere, arranged jointly by the American Phytopathological Society, the American Institute of Biological Sciences, and the Potato Association of America, at the 45th Annual Meeting of the American Phytopathological Society in Madison, Wisconsin.

Publication No. 198 del Instituto de Fitotecnia, Dirección General de Investigaciones, Ministerio de Agricultura y Ganadería.

[2] Director of the Instituto de Fitotecnia, Castelar, Argentina.

[3] The author is indebted to E. C. Stakman, J. A. Rupert, M. Bastos Lagos, Emilio Rojas M., D. Volosky de Hernandez, E. Godoy and co-workers, who furnished unpublished data that have made possible a more complete summarization on physiologic specialization of the wheat rust pathogens.

Almost all the samples identified as race 15 were 15B. However, race 15, as well as many others, should be considered as groups of biotypes.

In recent years, race 15 has become abundant in some areas of Argentina and Brasil and especially in Chile where it is at present the most widespread race. Race 17 is still the most common in Argentina and Brasil, and recently has become so in Peru where it

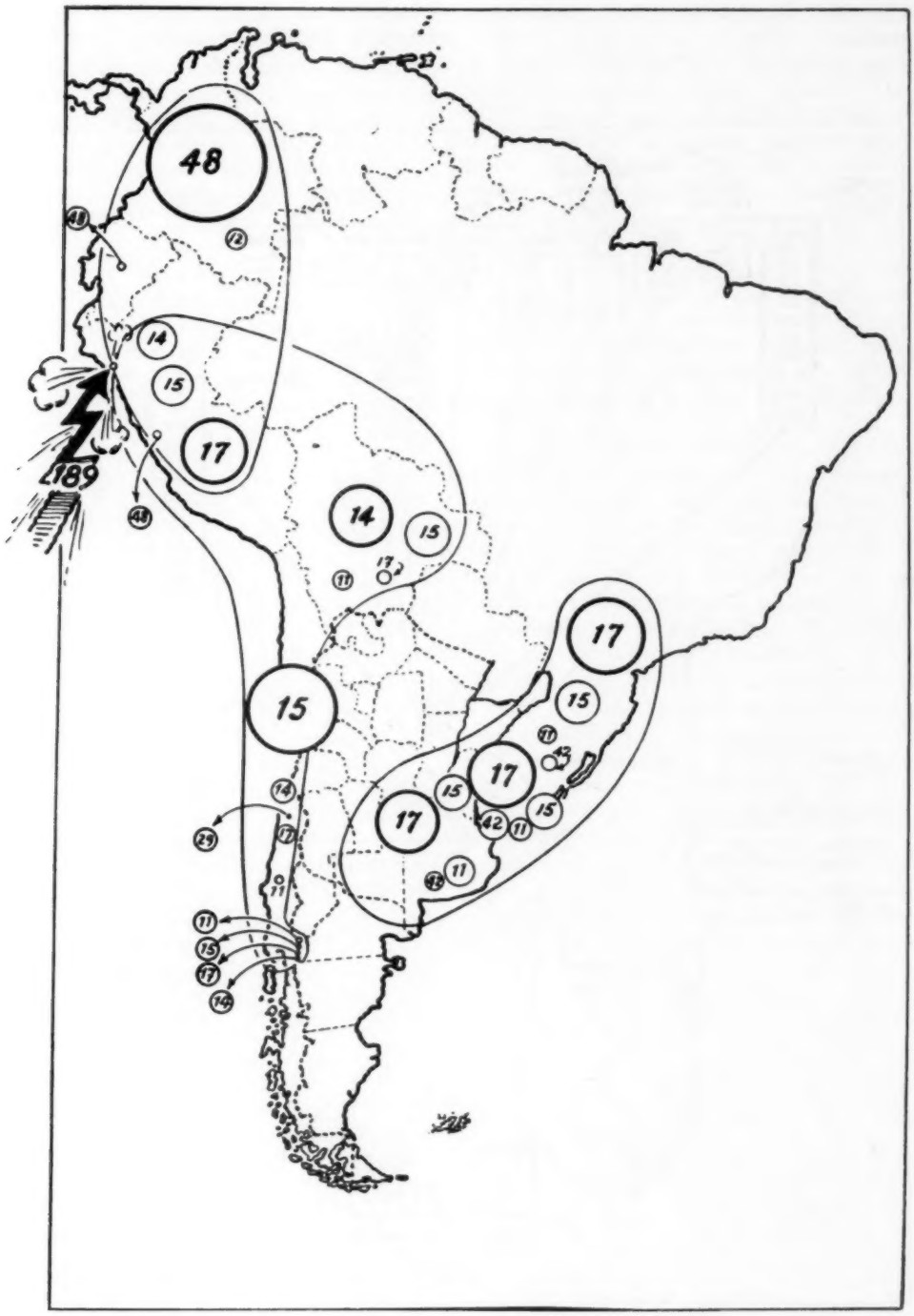


FIG. 1. Distribution of races of *Puccinia graminis* f. sp. *tritici* in South America and the suggested Atlantic and Pacific regions of race distribution.

was found first in 1949. The South American biotypes of race 17 possess great aggressiveness, especially on *T. timopheevi*, *T. durum* and many common wheats (Egypt N.A.95, Frontana and Magnif Fandsoon). Race 42, formerly prevalent in Argentina, Uruguay, and Brasil, and race 14 in Chile and Peru, have decreased markedly. Race 48 is still the most common in Colombia.

Considering South America as a whole, there appear to be 2 main regions (Fig. 1): the Atlantic coastal area, characterized by the presence of race 42, which is decreasing in prevalence because of the resistance of the wheat varieties now grown; and the Pacific coastal area, characterized by the presence of races 48 and 14, the first prevalent in the northern, the second in the southern part. Other races occur in

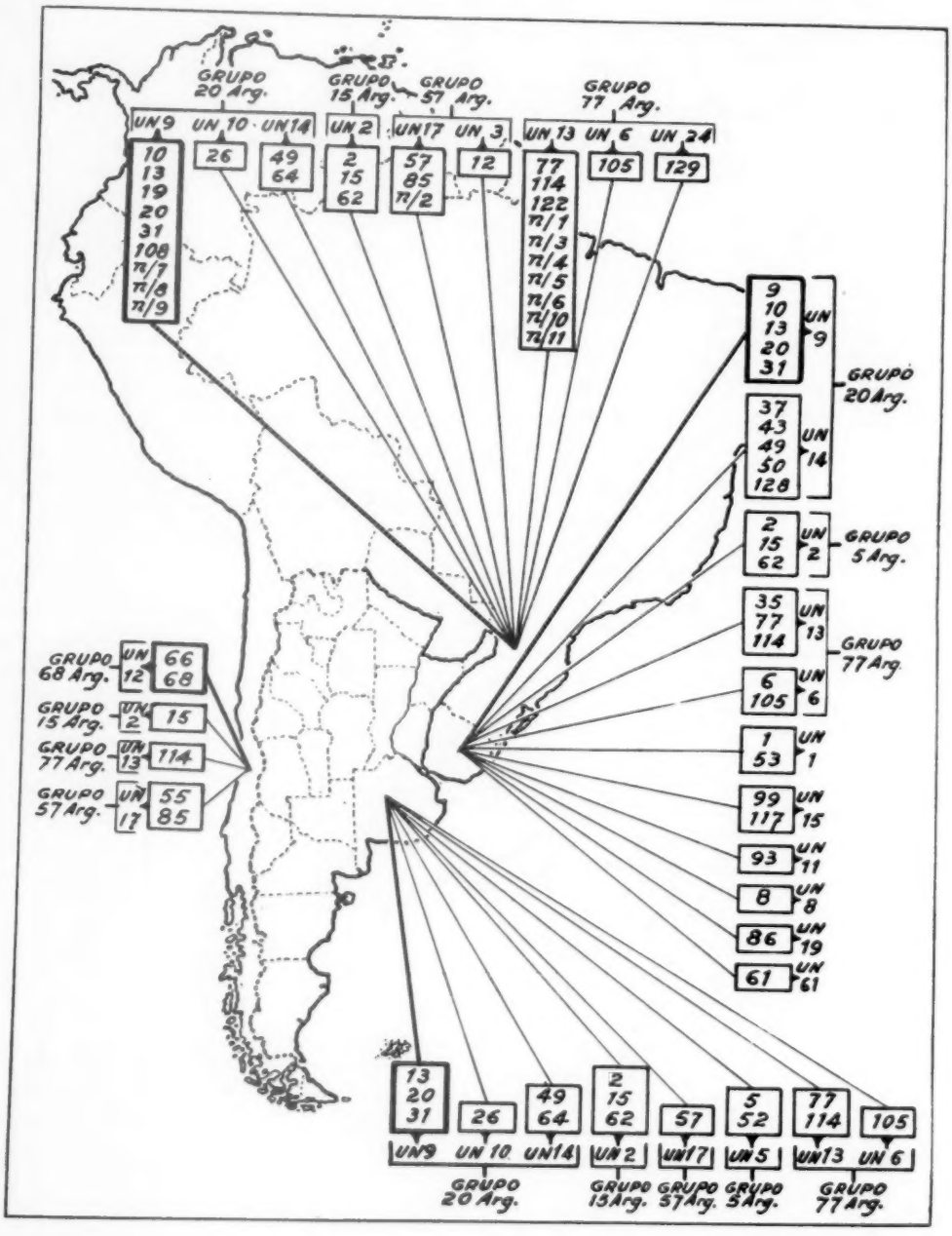


FIG. 2. Distribution of races of *Puccinia triticina* in South America. The races are indicated under each of the 3 systems of classification: Johnston and others, unified numeration, and Argentine grouping of races.

these 2 main areas and some of them are designated as 17, 15, and 11, although probably not always the same biotypes of these races.

It has been shown also that, at least in the southern part of the Andean Mountains, the races from the Pacific area can be blown to the Atlantic area through the Patagonian Valleys, although it is unknown whether they are distribtuted northward into the main cereal regions of the Atlantic area.

There is no experimental evidence in South America that the barberries play a role in the spreading of stem rust or in the production of new races. Nevertheless, the possibility cannot be discarded that in some locations and under special conditions they may be a source of new races. This appears to be true in Peru and Colombia.

PUCCINIA TRITICINA.—Many races of *P. triticina* have been identified in South America, the number depending upon the method used for their classification (Fig. 2). The summary of current information using the 3 more common race designations follows: In Chile, races 66[4] and 68 (= UN 12[5]); 15 (= UN 2); 114 (= UN 13); 55 and 85 (= UN 17); in Brasil races 10, 13, 19, 20, 31, 108, n/7[6], n/8, n/9, (= UN 9); 26 (= UN 10); 49, 64 (= UN 14); 2, 15, 62 (= UN 2); 12 (= UN 3), 77, 114, 122, n/1, n/3, n/4, n/5, n/6, n/10, n/11 (= UN 13); 105 (= UN 6); 129 (= UN 24); 57, 85, n/2 (= UN 17); in Uruguay 9, 10, 13, 20, 31 (= UN 9); 37, 43, 49, 50, 128 (= UN 14); 2, 15, 62 (= UN 2); 35, 77, 114 (= UN 13); 6, 105 (= UN 6); 1, 53 (= UN 1); 99, 117 (= UN 15); 93 (= UN 11); 8 (= UN 8); 61 (= UN 3); and 86 (= UN 19); in Argentina 13, 20, 31 (= UN 9); 26 (= UN 10); 49, 64 (= UN 14); 2, 15, 62 (= UN 2); 57 (= UN 17); 5, 52 (= UN 5); 77, 114 (= UN 13); and 105 (= UN 6) (Fig. 2).

In Argentina, races UN 9, UN 10, and UN 14 are assigned to a single group (Arg. 20) since they are all unable to attack wheat varieties carrying the Sinvalocho factors for resistance. This group of races is prevalent in Argentina, Uruguay, and Brasil. Races UN 2 (group Arg. 15), UN 13, UN 6, and UN 24 (group Arg. 77) all highly virulent on Sinvalocho together with some unusual biotypes of group Arg. 20 that attack Frontana, also are spreading in an alarming manner in this area. In Chile, 68 (UN 12) is still the prevalent race.

The change in race population and its relation to the reaction of the cultivated wheats and of those used in breeding are shown best by grouping the races according to their reaction to the specific genetic factors for resistance carried by the varieties. This regrouping of races is especially necessary with *P. triticina* which always shows a great and disconcerting variability. The use of additional differential varieties (Sinvalocho, Frontana, Buck Tandil, Klein Titán) for *P. triticina* has been necessary in Brasil and Argentina. During the survey made in the last named country in 1952, at least 5 biotypes of race 20 and 2 of race 15 have been found. The pathogenic behavior of some of these biotypes accounts for the unexpected reaction of some varieties previously regarded as resistant to the common races. Following the same concept, grouping for all of the rust species is being studied.

PUCCINIA GLUMARUM.—Very little has been done on the physiologic specialization of *P. glumarum* on wheat in South America. Rudorf and associates (10) found differences between the characteristics of the Argentinian and European races. Straib (15) identified races 30, 37, 38 and 39 in Chile, and race 30 in Argentina and Uruguay. All of these races as well as new ones, were found later in Argentina. None of these races is able to attack the variety Chino 166.

In recent years, Volosky de Hernandez has found in Chile a number of races showing a different pathogenic reaction on the Straib differentials and on South American wheat varieties. In contrast with the first mentioned races, especially those found in Argentina, almost all the races isolated by Volosky were capable of infecting Chino 166.

Our experience with *P. glumarum* shows that the use of a new set of differentials is necessary in order to evaluate the reaction of the cultivated wheat varieties and as a basis for the wheat breeding programs in South America.

INSTITUTO DE FITOTECNIA
CASTELAR, ARGENTINA

[4] According to key by Johnston and others (7).
[5] Unified numeration.
[6] New races described by A. Da Silva in Brasil: n/1, n/2, n/3, n/4, n/5, n/6, n/7, n/8, n/9, n/10, n/11.

LITERATURE CITED

1. ABBOT, E. V. 1929. Stem rust of wheat in Peru. Phytopathology **19**: 1041-1043.
2. BOASSO, C. S., AND M. N. LEVINE. 1951. Leaf rust of wheat, Puccinia rubigo-vera tritici, in Uruguay. Phytopathology **41**: 736-741.
3. FAVRET, E. A. 1947. Presencia de la raza 15 de "Puccinia rubigo-vera tritici" en la Argentina. Rev. Invest. Agr. Buenos Aires. **1**: 63-64.
4. GARCÍA-RADA, G., J. VALLEGA, W. Q. LOEGERING, AND E. C. STAKMAN. 1942. An unusually virulent race of wheat stem rust, No. 189. Phytopathology **32**: 720-726.
5. GODOY, E. F., H. J. SAVOIA, AND O. BRUNI. 1950. Importancia de la roya del tallo en 1949. In Memoria cuarto reunión de trigo, avena, cebada y centeno. Argentine Min. de Arg. y Ganad., p. 16-20.
6. GODOY, E. F., H. J. SAVOIA, AND O. BRUNI. 1950. La roya de la hoja en 1949. In Memoria cuarto reunión de trigo, avena, cebada y centeno. Argentine Min. de Agr. y Ganad., p. 21-31.
7. JOHNSTON, C. O., H. B. HUMPHREY, R. M. CALDWELL, AND L. E. COMPTOM. 1942. Third revision of the international register of physiologic races of the leaf rust of wheat (Puccinia rubigo-vera tritici

(triticina)). U. S. Dept. Agr. Bur. Pl. Indus. Div. Cereal Crops and Diseases, Washington. 20 p.
8. Revilla, V. A. 1945. Razas fisiológicas de la roya negra del trigo (Puccinia graminis tritici) encontradas en el Peru. Lima, Peru Estac. Exp. Agr. de La Molina Bol. 26, 16 p.
9. Rojas Mendoza, E. 1950. La roya negra del trigo en el Peru (Una nueva raza fisiológica la 17, se identifica para el país). Agronomía (Peru) **15**: 35-41.
10. Rudorf, G., M. M. Job, and K. V. Rosenstiel. 1933. Investigaciones sobre inmunidad en trigo. Univ. Nac. La Plata. Publ. Oficial.
11. Rudorf, G., and E. R. Langmann. 1953. Síntesis de las primeras investigaciones sobre Puccinia graminis tritici (Pers.) Erikss. et Henn. y Puccinia triticina Erikss. realizadas en el Instituto Fitotécnico de Santa Catalina. Notas Prelim. Museo La Plata. **2**: 65-75.
12. Silva, R. A. Da. 1947. Estudios preliminares para a produção de variedades de trigo resistentes as ferrungens no Brasil. Brasil Min. da Agr. Bol. Serv. Nac. Pesq. Agron. 1, 53 p.
13. Silva, R. A. Da. 1950. Melhoramento genetico das plantas cultivadas para resistença as doenças parasitarias. Principios fundamentais e sua aplicação aos trabalhos de melharamento do trigo. Tesis Prof. Esc. Agron. E. Maciel. Pelotas, Brasil.
14. Silva, R. A. Da. 1952. Raças fisiológicas de ferrugem da folha do trigo no Brasil e a reação de algumas variedades de trigo a 14 rações. 2a. Reun. Lab. Am. Fitog.
15. Straib, W. 1937. Las razas fisiológicas de Puccinia glumarum en Sud América y su comportamiento en la infección comparado con el de las formas europeas. Arch. Fitotécn. Uruguay. **2**: 217-233.
16. Vallega, J. 1937. (1938). Dos nuevas razas seleccionadas de trigo de origen híbrido inmunes a Puccinia glumarum. Rev. Facult. Agron. (La Plata) **22**: 139-145.
17. Vallega, J. 1940. Especialización fisiológica de Puccinia graminis tritici en la Argentina, Chile y Uruguay. Rev. Argentina Agron. **7**: 196-220.
18. Vallega, J. 1941. Razas fisiológicas de "Puccinia triticina" procedentes de Ipanema, San Pablo, Brasil. Rev. Argentina Agron. **8**: 57-59.
19. Vallega, J. 1941. (1943). Especialización fisiológica de Puccinia graminis tritici en Brasil. An. Inst. Fitotécn. Santa Catalina **3**: 29-36.
20. Vallega, J. 1942. (1944). Razas fisiológicas de Puccinia rubigo-vera tritici comunes en Argentina. An. Inst. Fitotécn. Santa Catalina **4**: 40-58.
21. Vallega, J. 1942. Razas fisiológicas de Puccinia tritici y P. graminis tritici comunes en Chile. [Chile] Min. Agr. Depto. Genét. Fitotécn. Bol. Técn. 3, p. 1-33.
22. Vallega, J. 1947. Reacción de algunas especies espontáneas de "Hordeum" con respecto a las royas que afectan al trigo. Rev. Invest. Agr. Buenos Aires **1**: 52-62.
23. Vallega, J. 1948. Razas de las royas de los cereales halladas en el archipiélago de Chiloé. Lilloa **21**: 169.
24. Vallega, J. 1951. Consideraciones sobre la raza 15B de Puccinia graminis tritici. Idia (Inform. Direc. Invest. Agr. Buenos Aires) **4**: (45): 14-17.
25. Vallega, J., H. P. Cenóz, E. Favret, J. A. Sarasola, J. L. Tessi, N. Horovitz, C. Cfalzeta, J. H. Frecha, and P. Rodriguez Amieba. 1951. Epifitia de la roya del tallo en los cultivos de trigo en 1950. Min. Agr. Div. Inm. Veg. Buenos Aires, Hoja Informe. 13, 3 p.
26. Vallega, J., H. B. Cenoz, J. L. Tessi, and J. H. Frecha. 1953. Observaciones realizadas durante el año 1952 en el registro epifitológico y en el ensayo territorial de resistencia a las enfermedades de los cereales. Min. Agr. Div. Inm. Veg. Buenos Aires, Hoja Informe. 14.
27. Vallega, J., and E. A. Favret. 1947. Razas fisiológicas de "Puccinia graminis tritici" que atacan a "Triticum timopheevi". Rev. Invest. Agr. Buenos Aires. **1**: 113-118.
28. Vallega, J., and E. Favret. 1952. Las royas de las cereales en Argentina. I. Caracteristicas patógenas de las distintas especies de royas. Idia (Informe. Direc. Invest. Agr. Buenos Aires) **5** (54): 17-39.
29. Vallega, J., and E. A. Favret. 1947. Royas y otros parásitos de los cereales en los valles andino-patagónicos. Rev. Invest. Agr., Buenos Aires. **1**: 269-277.
30. Volosky, D. 1945. Identificación de razas fisiológicas de Puccinia graminis tritici y P. triticina. Algunos estudies efectuados en Chile. Agr. Técn. Chile **5**: 37-47.
31. Volosky, D. 1946. Nuevas razas fisiológicas de Puccinia triticina para Chile. Agr. Técn. Chile **6**: 67.

SPECIES OF SOLANUM (TUBERARIUM) OF SOUTH AMERICA PRESENT TAXONOMIC STATUS AND SPECIES USED IN PLANT BREEDING WITH SPECIAL REFERENCE TO PERU [1]

C. Ochoa[2]

Several expeditions have been made, principally by Europeans, for the purpose of collecting native South American potatoes, but there still remain many unexplored areas, and there are many species which still need to be studied (7, 12). Unfortunately no country of South America has been interested until now in making a complete collection and in using the collection effectively in a potato breeding program. No experimental station in the world has a living collection that comprises even one-half of the wild species of South America. Many such species are known only as herbarium material which has been disseminated to different herbaria throughout the world. Some types formerly in European herbaria were lost during the last World War; the types and isotypes collected and described by the Soviet investigators are not now available; other species are extremely rare. Many of the descriptions were based on material grown in the research institutes of England or Russia. All this naturally has created a chaotic situation in the actual classification of the South American potatoes and makes necessary a complete revision.

This sparsity of exact knowledge of the native material explains in part the slow advance made in potato breeding in the Andean countries. More than 160 wild species of *Solanum* (*Tuberarium*) from South America have been classified. Only about 30 per cent have been studied by the plant breeders. The majority of investigations have been conducted in Europe or in the United States. The present paper is a brief discussion of the geographic distribution of the potato species of South America, the results obtained in potato breeding with special reference to Peru, and the possibilities for coordinated investigations in the future.

WILD SPECIES.—Sources of resistance to certain types of virus have been found in some species of the Chilean series *Etuberosa*, and observed in *Solanum simplicifolium*, a wild species from the northwest of Argentine (14). In general the sources of resistance to *Phytophthora infestans* have been unsatisfactory (1, 3, 4, 6, 8, 14, 18, 19). However, the relatively small number of species studied for resistance in comparison with the total number reported suggests that species will be found that are immune or highly resistant to attacks by this fungus. Some reports indicate such possibilities, for example, *S. andreanum* from Colombia (14) and *S. capsicibaccatum* from Bolivia (2) have shown resistance to late blight.

Among the South American countries, Peru represents the most important area of diverse genetical material. About 38 per cent of the South American wild potatoes are found in this country. Based on the study of the total number of *Solanum* (*Tuberarium*) found to date in the Americas, the species from Peru are more significant perhaps than those from Mexico. Mexico has been explored extensively and the quantity and diversity of material as well as the numerous species resistant to or immune from races of *Phytophthora infestans* are encouraging. At present, 43 species and varieties have been described from Mexico; whereas 58 species, without considering varieties, have been reported from Peru (5, 10, 12, 15, 16, 21). Furthermore, several zones in Peru are unexplored.

Wild potatoes in Peru are distributed principally in the highlands between 3000 and 4500 m above sea level. Some of them are noteworthy for their great resistance to frost injury. Other species occur in zones between 1000 and 2500 m where the climate is humid and the rainfall heavy. Along the coast at a few meters above sea level where the rainfall is very light, a number of other species grow in an arid environment. These species must be subjected to a detailed study of their anatomy, morphology and physiology in exploring their potential in the breeding of new varieties resistant to drought.

CULTIVATED SPECIES.—This group is represented exclusively by the series *Tuberosa* in which known species form a polyploid series (2n=24, 36, 48 and 60 chromosomes). The tetraploids *S. tuberosum* and *S. andigenum* are most important not only because of their valuable economic characteristics, but also because of their wide geographic adaptation, as represented by the great number of varieties and forms studied to date. Hawkes (13) proposed the division of *S. tuberosum* into 2 subspecies: 1) *andigenum*, represented by the cultivated varieties in the Andean region between southern Venezuela and northwest Argentine, and 2) *chileanum*, which is native to the Islands of Chiloe and to the coast of Chile. According to Russian workers, *S. tuberosum* represents the origin of all the varieties cultivated today in Europe (5), but Hawkes (12) and others suggest *S. andigenum*.

At present, no variety of *S. tuberosum* or *S. andigenum* highly resistant to late blight has been found. Some clones of *S. andigenum* have been highly resistant to *Synchytrium endobioticum* (15), the cause of a disease widely distributed in the Andes. The most

[1] Invitational paper presented September 9, 1953, at the Symposium on Cooperative Agricultural Research in the Western Hemisphere, arranged jointly by The American Phytopathological Society, The American Institute of Biological Sciences, and The Potato Association of America, at the 45th Annual Meeting of The American Phytopathological Society in Madison, Wisconsin.

[2] Division de Investigacion y Experimentacion Agricola. Ministerio de Agricultura, Lima, Peru.

important contribution of these species to potato improvement probably is their high yielding ability.

The cultivated diploid group has been found endemic only in Colombia, Ecuador, Peru, and Bolivia. Among the Peruvian species of this group are: *S. goniocalyx*, a potato of high quality, *S. churuspi*, *S. yabari* and *S. stenotomum*. These species are widely distributed in southern and central Peru. *S. stenotomum* represents 1 of the best available sources of such desirable economic characteristics as earliness, yield, quality, and resistance to frost injury. Some clones of this species showed good combining ability in intraspecific and interspecific crosses. Among the diploids from Bolivia are *S. ajanhuiri*, resistant to frost injury; *S. cardenasii* and *S. phureja*, early and palatable. With the apparent exception of *S. ajanhuiri*, all of the diploid species mentioned are susceptible to the complex of virus diseases.

The triploid species are confined to Peru and Bolivia. The triploid species *S. coeruleiflorum*, *S. mamilliferum* and *S. choclo* are endemic to Peru. Three species occurring in both countries are: *S. juzepczukii*, with a bitter flavor, but with resistance to frost injury; *S. chaucha*, the earliest native cultivated species (90 days); and *S. tenuifilamentum*. The last 2 have good flavor, but are low in yielding ability.

The only pentaploid cultivated species found to date is *S. curtilobum*, common to Peru and Bolivia. It is resistant to frost injury, but has a bitter flavor. The species is highly self fertile, and fertile in crosses with *S. andigenum* especially where the latter species is used as the pollen parent. *S. curtilobum* and the triploid *S. juzepczukii* constitute the main source of the native "*chuno*" or dehydrated potato.

The little known Peruvian cultivated species *S. triferum* (17, 20) appears to be nearly extinct. After years of search for material in the tropics of Peru, it has been found cultivated under the native name "*Curahui*" at an altitude of 500 to 700 m above sea level and in a very reduced area. This species has not been studied in detail.

BREEDING WORK WITH SPECIAL REFERENCE TO PERU.—Recently a few South American countries have been interested in potato breeding. Chile began work on cultivated potatoes in 1941 (13). Colombia began with a broader program in 1948, organized by the English scientist J. G. Hawkes. The material being studied at present comprises practically all of the potatoes from Colombia, many of the species from Central American countries, and some of the species from other parts of South America. Argentina has organized 2 expeditions to Chile to broaden potato breeding work; the first was in 1938 under the direction of J. Vallega and L. de Santis (8); the second, in 1948, was headed by Alfonso Castronovo (9).

In Peru the first breeding work started about 1930, but it was discontinued for many years. In 1946 the program was revived and it has been continued with investigations on yield, resistance to frost injury, and resistance to late blight as summarized in the following paragraphs.

Breeding for yield.—The most widely cultivated varieties in Peru are the result of empirical selections made by farmers who were interested in obtaining higher yields and more uniform products. An attempt was made by the commercial growers to replace the conglomerate of forms, which had been cultivated for many centuries, with a more uniform potato. Beginning in 1946, the author has made collection trips to the most distant regions of the Peruvian Andes. Hundreds of samples were collected, arranged in taxonomic order, and subsequently maintained in a living condition at the Experimental Station of Huancayo at 3300 m above sea level. Comparative tests on yields, using modern experimental techniques, were made to determine the best material for crossing. High yielding lines were used to produce the first hybrids. Selections from these showed high yielding ability in comparison with the best native varieties previously tested and selected.

The best selections to date were from hybrids derived from intervarietal and intravarietal crosses of *S. andigenum*, notably varieties *juninum*, *platyantherum*, *ancashicum*, *tumbo*, and others. Among interspecific crosses the best results were obtained with progenies from *S. curtilobum* × *S. andigenum*. Yield increases of 25-30 per cent were obtained in comparison with the indigenous varieties "Yana marca" = ("Casa Blanca") and "Jiruco" which were the 2 highest yielding varieties in the previous comparative investigations. The varieties "Renacimiento" and "Pachacutec" originated from these crosses and their multiplication is in progress.

Breeding for resistance to frost injury.—In all Andean countries, frost damage is a serious problem unsolved in potato production. A variable range of resistance to frost damage has been reported in the wild South American species *S. acaule*, *S. bukasovii*, *S. abbottianum*, and *S. neohawkesii* from Peru; *S. rionegrinum*, *S. commersonii*, *S. depexum*, *S. millanii* and *S. chacoense* from Argentina; *S. henryii* and *S. ohrondii* from Uruguay; and *S. brevicaule* from Bolivia (5, 11, 12, 14, 15). New species found by the author have shown resistance to frost injury at −3°C; these are *S. sogarandinum* and *S. jalcae* in the *Megistacroloba* series. Likewise, *S. acaule* var. *albicans* of the *Acaulia* series was not damaged by temperature as low as −5°C. Tests made in selections from other species such as *S. pumilum*, *S. raphanifolium*, *S. multidissectum* and *S. canacense* have shown variable resistance to frost damage between −2°C and −3°C.

Most of the Peruvian species mentioned have been used in crosses with cultivated varieties. The most promising results were obtained from hybrids of *S. andigenum* × *S. acaule* and *S. acaule* × *S. stenotomum*. Selections from these hybrids showed resistance to −3°C, but these plants gave small tubers of bitter flavor and they had stolons more than 1 meter in length. Subsequently back crosses were made to

eliminate these unfavorable factors, and although the new lines were better than the parents in yield and quality, they were considerably less resistant to frost injury.

Among the previously mentioned frost-resistant material, the diploid *S. neohawkesii* appeared very valuable because of its high combining ability. Crosses with *S. yabari* and *S. stenotomum* were 60 per cent successful, and crosses with *S. goniocalyx* were over 85 per cent successful. These hybrids were highly fertile and some of them were fertile in crosses with the tetraploid group. Some lines from *S. goniocalyx* × *S. tuberosum* showed good tuberization, agreeable flavor, acceptable yield and resistance to −1° and −2°C.

An interesting variety from *S. stenotomum*, named var. *mauna*, was found as resistant to frost damage as *S. curtilobum*, and it was better in quality than many of the frost resistant species. This variety when crossed with *S. andigenum* gave fertile hybrids. Lines selected from this cross gave medium yield, good flavor, and resistance to −2°C, but these selections showed a very prolonged vegetative period.

Crosses of *S. curtilobum* with tetraploid clones have resulted in lines with good yielding capacity, good quality and medium resistance to frost injury.

All attempts to obtain hybrids between *S. juzepczukii* and the cultivated species of the polyploid series have failed. This species has a greater resistance to frost damage than does *S. curtilobum* and *S. ajanhuiri*, and although it flowers abundantly, it does not produce seed. Pollen analysis showed 1.5 per cent of fertile pollen.

Breeding for resistance to late blight. — Using Black's differentials, Bazan de Segura (4) has identified the presence of races A, C, and D of *Phytophthora infestans* in Peru. Dr. W. Black has found recently 2 new races of the pathogen designated I and J from this country.[3]

Inoculations on varieties of *S. andigenum*, wild species, and hybrids were conducted by Mrs. Segura in order to determine the reaction to late blight (3, 4). All samples of *S. andigenum* tested were susceptible, *S. juglandifolium* (from Colombia) was immune to race C, *S. ochranthum* (from Peruvian collection) was susceptible to C, and *S. chacoense* (from Argentina) was susceptible to C and D. Of 4 samples of undetermined wild Peruvian species, 1 was immune to C, another was immune to D, and 2 were susceptible to C.

Promising hybrids have been obtained at La Molina Experimental Station from the Mexican species *S. antipoviczii* in crosses with an intraspecific hybrid of *S. andigenum*. When artificially inoculated, F_1's of these hybrids have shown immunity to races A, C, and D (4).

Field inoculations with the common race were made by the author at the Experimental Station of Huancayo on some hundreds of cultivated forms and wild species. Four Mexican species, *S. bulbocastanum*, *S. pinnatisectum*, *S. demissum*, and *S. antipoviczii*, reported immune, were used as comparative material. Our results show immunity for *S. bulbocastanum* and *S. pinnatisectum* and susceptibility for *S. demissum* and *S. antipoviczii*. Crosses were tried between the 2 immune and cultivated species without success.

The late blight reaction of the cultivated species was of the susceptible type in all varieties tested. *S. yabari*, *S. tenuifilamentum*, *S. mamilliferum*, *S. chaucha*, *S. coeruleiflorum*, and *S. goniocalyx* were very susceptible. Different degrees of susceptibility were observed in the clones tested of *S. stenotomum*, *S. andigenum*, and *S. curtilobum*. The wild species, *S. hypacrarthrum*, *S. gracilifrons*, *S. immite*, *S. raphanifolium*, *S. neohawkesii*, *S. rhomboideilanceolatum*, *S. weberbauerii*, *S. neoweberbauerii*, *S. wittmackii*, *S. medians*, *S. multiinterruptum*, *S. acroscopicum*, *S. abbottianum*, *S. bukasovii*, *S. arac-papa*, *S. acaule*, and *S. ochranthum* were all susceptible. However, *S. chiquidenum* showed the presence of very small necrotic flecks, indicating a high type of resistance.

COOPERATIVE INVESTIGATIONS ON AN INTERNATIONAL BASIS.—The South American native species of *Solanum* (*Tuberarium*) offer great possibilities for use in a breeding program. Breeding for disease resistance will be most effective only through cooperation on an international basis. Problems with the potato crop are essentially similar in the majority of the South American countries. Virus diseases and late blight represent similar problems wherever found. The following phases of the problem are suggested for this cooperative program:

1) Immediate support for maintaining the presently known Central and South American potato germplasm in living condition at a central station in Peru and elsewhere. 2) Exhaustive collecting expeditions conducted by experienced potato taxonomists and pathologists in all of the known centers to secure new sources of germplasm and information on potato diseases. 3) Assembling a staff of trained scientists in several centers under a fully coordinated plan to grow and study the material including indexing for disease. 4) Preparation and study of comprehensive herbaria of *Solanum* and related genera for world wide distribution. 5) Production of seed and tubers for the safe distribution of the material to the various investigators interested in potatoes. 6) Coordination of basic and applied research to insure maximum efficiency in the use of material and information for potato improvement.

HUANCAYO, PERU

[3] Personal communication.

LITERATURE CITED

1. ANONYMOUS. 1940. Comm. Agr. Bur. Ann. Rpt. Exec. Counc. (**1938-39**): 52-54.

2. Anonymous. 1951. Comm. Agr. Bur. Ann. Rpt. Exec. Counc. (**1950-51**): 32-33.
3. Bazan de Segura, C. 1951. I. Trabajos preliminares para la obtencion de variedades de papa resistentes al "hielo" (Phytophthora infestans) en el C. N. I.E.A. de La Molina. II. Reaccion de las variedades de papa peruanas al "hielo" (Phytophthora infestans (Mont.) de Bary). Argentine Min. de Agr. Centro Nac. Invest. Exp. Agr. La Molina, Lima, Bol. 43, 24 p.
4. Bazan de Segura, C. 1952. I. Razas fisiologicas de Phytophthora infestans en el Peru. II. Investigaciones sobre resistencia de especies, variedades e hibridos de papa al P. infestans. Argentina Min. de Agr. Centro Nac. Invest. Exp. Agr. La Molina, Lima, Bol. 46, 16 p.
5. Bukasov, S. M. 1933. (The potatoes of South America and their breeding possibilities) Bul. Appl. Bot., Genet., and Pl. Breeding, Leningrad. Suppl. 58, 192 p. (In Russian, English summary).
6. Bukasov, S. M. 1936. The problems of potato breeding. Amer. Potato Jour. **13**: 235-252.
7. Cardenas, M., and J. G. Hawkes. 1945. New and little-known wild potato species from Bolivia and Peru. Linn. Soc. London Jour. **53**: 91-108.
8. Castronovo, A. 1949. Papas Chilotas. Descripciones y clave para el reconocimiento de muestras de papa recogidas en una excursion al Sur de Chile. Rev. Invest. Agr., Buenos Aires **3**: 209-246.
9. Castronovo, A. 1950. Fuentes de resistencia a "Phytophthora infestans". Rev. Invest. Agr., Buenos Aires **4**: 245-275.
10. Correll, D. S. 1952. Section Tuberarium of the genus Solanum of North America and Central America. U. S. Dept. Agr. Agr. Monog. 11, 243 p.
11. Estrada, N. 1953. La resistencia a las heladas en relacion con varias especies de papas. Agr. Tropical, Bogota, **5**: 17-22.
12. Hawkes, J. G. 1944. Potato collecting expeditions in Mexico and South America. II. Systematic classification of the collections. Imp. Bur. Plant Breeding and Genet., Cambridge. 142 p.
13. Hawkes, J. G. 1950. Conferencia Latino Americana de Especialistas de papa. Min. Agr. Ganad., Div. Agric., Est. Exp. Papa, Bogota, 78 p.
14. Hawkes, J. G. 1951. Cinco conferencias sobre el mejoramiento de la papa. Min. Agr., Inst. Nac. de Agr., Venezuela Bol. Tec. 1, 54 p.
15. Ochoa, C. 1951. Algunos estudios sobre papas peruanas como base para un programa de mejoramiento en el pais. Agronomia, Lima, **65**: 31-38.
16. Ochoa, C. 1952. Dos nuevas especies silvestres de papas diploides del Peru. Rev. Argentina Agron., Buenos Aires, **19**: 231-237.
17. Poepigg, E. F. 1835-36. Reise in Chile, Peru, und auf dem Amazonstrome während der Jahre 1827-1832. F. Fleischer, Leipzig. v. 2, 83 p.
18. Reddick, D. 1928. Blight-resistant potatoes. Phytopathology **18**: 483-500.
19. Reddick,D. 1939. Whence came Phytophthoria infestans? Chron. Bot. **5**: 410-412.
20. Schlechetendal, D. F. L. von. 1841. Hortus halensis. C. A. Schwetschke and Son, Halle, Saxony. Part 1, 8 p.
21. Vargas, C. 1949. Las Papas Sudperuanas (I Parte). Publ. Univ. del Cuzco.

LATE BLIGHT RESISTANCE—PRESENT STATUS [1]

L. E. Heidrick [2]

Late blight, caused by the fungus *Phytophthora infestans* (Mont.) d By., has been and undoubtedly still is the most serious disease affecting potatoes. The need for its control was first recognized after the potato famine of 1845; since that time, considerable progress has been made. The idea of using resistance to combat late blight may perhaps be traced to the observations by Jones and Stuart that some cultivated varieties of potato were not as heavily infected as others. Later, plant breeders began hybridizing these varieties to combine blight resistance with other desirable characteristics.

The search for resistance led investigators to Central and South America, where *P. infestans* is thought to have originated. In the belief that species growing in these regions would be resistant to the late blight pathogen, collections of wild potatoes were made in Mexico, Central America, and the Andean region of South America. An intensive program of hybridization was begun in both Europe and the United States with these collections. Plant breeders soon discovered, however, that although several of the wild species were resistant or immune, only one of these, *Solanum demissum*, could be used extensively; hybridization of the other resistant wild species with commercial varieties often resulted in pollen sterility, incompatibility, flower drop, and inheritance of the characteristics of the wild species. Nevertheless a few hybrids were developed which had greater resistance than the commercial varieties. This work yielded further the important information that resistant varieties might result from: 1) Selfing a susceptible variety, 2) Cross-

[1] Journal Series Paper No. 1 of the Colombian Agricultural Program of The Rockefeller Foundation. Invitation paper presented September 9, 1953, at the Symposium on Cooperative Agricultural Research in The Western Hemisphere, arranged jointly by the American Institute of Biological Sciences and the Potato Association of America on Cooperative Agricultural Research in the Western Hemisphere, Forty-fifth Annual Meeting of the American Phytopathological Society, held in Madison, Wisconsin, on September 9, 1953.

[2] Assistant Horticulturist with the Colombian Agricultural Program of The Rockefeller Foundation.

ing 2 resistant varieties, 3) Crossing a resistant and a susceptible variety, 4) Crossing 2 susceptible varieties, and 5) Crossing a resistant wild species with another variety.

Through the work of later investigators, some of these difficulties preventing hybridization were overcome in specific cases. It was found, for instance, that incompatibility between *S. demissum*, with 72 somatic chromosomes, and *S. tuberosum*, with 48 somatic chromosomes, could be overcome by the use of a bridging process. Thus, the bridge species *S. rybinii*, with a somatic chromosome number of 24, was crossed with *S. demissum* to produce an F_1 hybrid with 48 somatic chromosomes. This plant then was crossed with *S. tuberosum*. The resulting triple hybrid was fertile and could be used in additional back crossings to improve the commercial characteristics. This problem also was solved by using colchicine to double the chromosome number of a few species (*S. chacoense* and *S. bulbocastanum*) having 24 somatic chromosomes.

Progress also was made toward solving the problem of pollen sterility. Pollen sterility was explained as abnormal chromosome behavior or chromosome imbalance at the time of pollen formation, and attributed in triploids (*S. rybinii* 2n=24 × *S. ajuscoense* 2n=36) to the vegetative propagation of hybrids formed from irregular chromosome combinations. Further study then showed that most species of *Solanum* with an even multiple of the basic chromosome number (2n=24, 48, or 72) have a regular meiotic behavior and are easily hybridized. In the case of *S. chacoense*, self-sterility was eliminated by the method of dual pollination (first using pollen of *S. tuberosum* and then pollen of *S. chacoense*). Grafting on tomato (*S. tuberosum* on tomato-female × *S. polyadenium*-male) and application of low concentrations of 2,4-D to the flowers also resulted in fertile hybrids. Recently it has been shown that results of crossing many varieties can be predicted from their behavior in selfing.

While all of these methods are useful, their application is limited to individual situations. Progress in the production of resistant hybrids has been retarded by lack of understanding of both internal and external factors involved in the hybridization of the various species.

The breeding program in the past was developed without a thorough understanding of the biology of the pathogen. Recent developments indicate that it is necessary to take into consideration the variability of the pathogen in the breeding of resistant varieties. At present, it is known that a number of races of *P. infestans* exist in different parts of the world, that they are characterized by variability of pathogenicity or specialization, and that this variability, which is believed to be governed by gene mutations or other mechanisms within the organism, can be altered by external conditions. Present immune varieties developed in 1 area have proved susceptible in other areas, and at least 1 highly specialized race has been isolated from *S. demissum*, the chief source of resistance in the breeding program. In view of the variability of the pathogen *P. infestans*, it seems probable that such virulent races may eventually appear wherever resistant varieties are developed.

Since selecting for immunity within a given area is limited to the degree of specialization of the races present there, it is important that this work be done in an area where highly specialized races occur naturally or where they may be introduced and adequately controlled. Further investigation of the distribution of the various races and of the competition among them also should be made, and comparisons should be made of the reactions of the differential varieties in these areas.

The nature of the resistance of the plant is now being studied. Resistance has been attributed to solanin content, to peroxidase content, to hypersensitivity of the plant, and to many other factors. All of these factors are perhaps controlled by genes which may or may not be the same for the different degrees of resistance in plants or for the various races of the pathogen. Recent observations indicate that there is no definite relation between morphological characteristics of the plant or between weather conditions and resistance to late blight.

From the foregoing discussion, it is clear that many aspects of the late-blight problem require further study. For example, the limits of possible variations in hybridization should be analyzed. Comparisons should be made of the reactions of the various species and their hybrids in different areas with regard to seed formation, compatibility in interspecific crosses, and blight resistance. A study should be undertaken of the limitations of the production of interspecific and complex hybrids by the use of colchicine, bridging species, 2,4-D, and grafting with tomato. More must be known about the biochemistry of both the pathogen and the host. The effect of ecological factors on sterility in the potato should be investigated further. Studies of the effect of light, temperature, and humidity on the pathogen might help to explain the development and distribution of the races under different climatic conditions and the competition among them. These investigations would result in information of basic scientific importance as well as of commercial value.

As previously stated, the ease with which the virulence of the pathogen can be altered makes it unlikely that complete immunity can be maintained in a given variety over a long period of time. Breeding for partial or field resistance is therefore the answer to the late-blight problem, unless new factors of resistance can be secured. These must be sought simultaneously through a continuous breeding program and through investigation of the limits to hybridization of species, the biology and distribution of races of the pathogen, and of the nature of the resistance in the plant. Coordination of these activities is essential to further progress in blight resistance.

CONTROL OF DAMPING-OFF OF SUGAR BEET BY BACILLUS SUBTILIS[1]

John Dunleavy[2]

SUMMARY

An isolate of *Bacillus subtilis* (Cohn) Praz. from soil inhibited lateral growth of *Rhizoctonia* sp., the cause of a severe damping-off of sugar beet. A dilution of 10^{-3} of dried bacterial cells inhibited completely the growth of *Rhizoctonia* in culture. The antibiotic produced by the bacterium was more stable during autoclaving at pH 4.0 than at pH 7.0 and was not light sensitive. Although the antibiotic was produced on carbohydrate-free medium, its production in greater quantities requires the presence of a carbohydrate in the medium. The antibiotic was produced by the bacterium on water agar containing manure. The most active fraction of the antibiotic was extracted with n-butanol.

When *B. subtilis* was added to sterile soil inoculated with *Rhizoctonia* sp., the incidence of damping-off was reduced considerably in comparison with that which occurred in soil that did not contain the bacteria. High temperatures retarded inhibition of the fungus by the bacteria whereas low temperatures favored inhibition. The amount of manure in the soil determined the extent to which *B. subtilis* inhibited the *Rhizoctonia*. Limited control was achieved in non-sterile soil. The addition of a high nitrogen nutrient solution improved the control of damping-off of beet seedlings in non-sterile soil.

A number of instances have been reported of the control of fungus diseases of plants by the antagonistic activity of bacteria. Field tests conducted by Bamberg (3) showed that infection of leaves of corn plants by *Ustilago zeae* (Beck.) Ung. failed to occur when inhibiting bacterial organisms were present. Vasudeva (14) has found *Bacillus subtilis* effective in controlling pigeon-pea wilt caused by *Fusarium udum* Butl. Cordon and Haenseler (4) were able to control damping-off of cucumber and pea by adding *B. simplex* Gottheil to the soil. Sanford (10) reported control of potato scab by a number of different species of bacteria when green rye was applied to the soil. Porter (9) was successful in controlling flax wilt with an unidentified species of bacterium. Sanford and Broadfoot (11) were able to reduce the incidence of "take-all" disease caused by *Ophiobolus graminis* Sacc. with a number of different bacteria.

The objective of this study was to determine some of the factors influencing the antagonistic action of soil bacteria against a species of *Rhizoctonia*, which causes a severe damping-off of sugar beet. If antagonistic bacteria were found to exist in the soil, it was proposed to determine what environmental factors might influence the antagonistic relationship between the bacteria and the fungus.

MATERIALS AND METHODS.—The *Rhizoctonia* sp. was isolated from damped-off sugar beet seedlings and subcultured, using single hyphal tips. The white mycelium covered a 9 cm plate in 2 days and sclerotia were not formed in any of the 12 common media tested. All fungus inoculum used in the laboratory consisted of mycelium grown on 4 mm disks of potato dextrose agar.

Bacteria assayed for antagonism were isolated from soil clinging to the roots of sugar beet, wheat, and safflower plants. A suspension of soil from the plant roots was prepared. Dilution plates were then poured and kept at room temperature. Usually, in about 1 week fungi developed sufficiently in the plates to permit selection of antagonistic bacteria.

All bacterial colonies showing zones of inhibition were isolated and tested for inhibition of the *Rhizoctonia* by streaking bacteria across the center of a nutrient agar plate and by placing disks of agar containing the fungus at the edge of the plate opposite the bacterial streak (15). Bacterial inhibition of the fungus was determined by measuring the radius of the mycelial mats at right angles to the bacterial streak.

Unless otherwise stated, the bacterial isolate of *B. subtilis* used in all tests originated from a single colony and is designated as No. 65. Bacterial inoculum used in the laboratory was prepared by inoculating 50 ml of beef broth medium in a 200 ml Erlenmeyer flask with 1 ml of stock culture. Each culture was incubated at 35°C for 24 hours before use. The amount of inoculum used for all bacterial culture work was 0.25 per cent of the volume of the medium inoculated. Bacterial stock cultures were maintained in beef broth medium stored at 10°C.

To determine the concentration of antibiotic required to inhibit completely the growth of *Rhizoctonia* sp., a bacterial powder was prepared by inoculating 200 ml of a salt-asparagine nutrient solution, described by Jansen and Hirschmann (7), with *B. subtilis*. The cultures were incubated at 35°C in the dark for 4 days, then centrifuged at 2,600 rpm for 10 minutes. The supernatant liquid was poured off and the bacterial debris dried on a glass plate at room temperature. The dried residue was ground to a finely divided powder in a mortar. Yields averaged slightly less than 1 g of powder per liter of culture.

Bacterial inoculum used in the greenhouse work was prepared by growing *B. subtilis* in petri dishes on

[1] Accepted for publication December 13, 1954.
Published with the approval of the Director as Paper No. 655, Journal Series, Nebraska Agricultural Experiment Station.

[2] Present address: Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa.

salt-asparagine medium containing 1.7 per cent agar. The plates were seeded with 1 ml of bacterial inoculum and incubated at 35°C for 24 hours. Bacteria were washed from the plates with distilled water and the washings from 30 plates were diluted to 1 liter with distilled water.

Fungus inoculum used in greenhouse tests was prepared by growing the *Rhizoctonia* on boiled and autoclaved corn seed for 5–7 days.

Five-in. clay pots were used throughout the greenhouse tests. Nonamended soil used was Marshall silty-clay loam. Greenhouse soil was composed of 3 parts Marshall silty-clay loam, 1 part sand, and 1 part manure. Soil to be inoculated with bacteria was removed from the pot and 30 ml of inoculum thoroughly mixed with the soil. Soil was inoculated with the *Rhizoctonia* by placing 5 kernels of corn, on which the fungus was growing, 1 in. below the surface of the soil.

Sugar beet seed used throughout the study was a selection designated as 526, which is susceptible to *Rhizoctonia* root rot. Ten seeds were planted per pot of soil at a depth of ¼ in.

RESULTS. — *Properties of the antibiotic.* — Bacteria that inhibited fungi on dilution plates were assayed for their antagonism to *Rhizoctonia* sp. by means of the bacterial streak method. Width of mycelial mats was recorded for each of 65 bacterial isolates tested in triplicate. Classified according to degree of inhibition produced, 30 isolates showed good inhibition, 10 fair, 16 poor, and 9 no inhibition.

The best inhibition of *Rhizoctonia* sp. was displayed by 5 isolates, which were identified as *B. subtilis*. The majority of the isolates showing good inhibition of the fungus were of the same cultural type as the identified isolates and presumed to be the same species.

To find the concentration of antibiotic necessary to inhibit completely the growth of *Rhizoctonia* sp., a powder was prepared consisting primarily of dried bacterial cells. Quintuplicate agar plates of concentrations 10^{-3}, 10^{-4}, and 10^{-5} of the bacterial powder in salt-asparagine agar of pH 4.0 were prepared and inoculated with the fungus.

No perceptible growth of the fungus occurred on the agar plates containing a concentration of antibiotic of 10^{-4} for 48 hours. After this initial lag, the growth of the fungus on these plates approximated the growth on plates containing a concentration of the antibiotic of 10^{-5}. After 6 days, the lateral growth of *Rhizoctonia* sp. on agar containing the antibiotic at a concentration of 10^{-5} was reduced about 25 per cent and at a concentration of 10^{-4} about 50 per cent when compared with the control plates. No growth of the fungus occurred at a concentration of 10^{-3}.

To determine whether the inhibition of *Rhizoctonia* sp. was due to the presence of the bacteria themselves or to the secretion of some substance into the medium, the bacteria were removed from a 10-day old culture in salt-asparagine medium with a Seitz filter and the filtrate tested for the presence of the antibiotic. There was little inhibition of lateral growth of the fungus on nutrient agar containing the filtrate, although the mycelial mat was thinner than on control plates. Bacterial cells from 10 ml aliquots were washed 3 times and resuspended in 10 ml of water. Six portions of 10 ml each of resuspended bacterial cells were combined. The suspension was divided into 2 30-ml portions and 1 of these was autoclaved twice to kill the bacterial spores. Quintuplicate agar plates were prepared for assay by diluting 3 ml of the suspension with 7 ml of potato dextrose agar. The plates were inoculated with *Rhizoctonia* sp. Suppression of lateral growth of the fungus was used to indicate the degree of inhibition as compared to growth on control plates which contained no bacterial cells. Lateral growth of the fungus was quite suppressed indicating that a considerable amount of antibiotic had been retained by the bacterial cells. The second portion of the suspension was steamed for 1 hour twice daily on 3 successive days. The suspension was assayed just as the first portion and there was no difference in antibiotic potency between the steamed and autoclaved preparations.

Subtilin has been reported to lose antibiotic activity when autoclaved for 10 minutes at 10 lb. of pressure at pH 7.0 but retains its activity when the pH is well on the acid side (7). In order to compare the stability of the antibotic under study with that of subtilin at high temperature, the following experiment was carried out. One-half of a 4-day old culture of *B. subtilis* in salt-asparagine medium was adjusted to pH 7.0 and dispensed in 100 ml quantities to each of 5 flasks. The remainder of the culture was adjusted to pH 4.0 and likewise dispensed to 5 flasks. Cultures were autoclaved at 10 lb. pressure for 10 minutes and suspensions of dead bacterial cells were assayed for antibiotic activity by diluting 1 ml of the suspension with 9 ml of potato dextrose agar in petri dishes. Control plates contained 1 ml of sterile medium of the appropriate pH instead of the bacterial suspension. The plates were inoculated with *Rhizoctonia* sp. and the radius of the colonies recorded after 24 hours. The average colony radius on plates containing the bacterial suspension at pH 4.0 was 1.4 mm and that at pH 7.0, 4 mm. Control plates averaged 6 mm in radius and showed no significant change in lateral growth due to the addition of 1 ml of sterile medium at pH 4.0 instead of pH 7.0. It was concluded that, as is the case with subtilin, more antibiotic activity is retained if the pH of the bacterial suspension is well on the acid side during autoclaving.

Jansen and Hirschmann (7) have described a color test for subtilin activity. When this was applied to several isolates of *B. subtilis*, isolates showing weak antibiotic activity against *Rhizoctonia* sp. gave weak tests whereas those having high antibiotic activity gave strong tests. Isolate No. 65 gave a particularly strong test. The intensity of color in this test also varied with the medium used. Intensity was always

greatest in media that supported maximum antibiotic activity and less in other media.

Subtilin has been reported to be light sensitive (7). To determine whether the antibiotic produced by isolate No. 65 is light sensitive, 5 freshly inoculated cultures of *B. subtilis* in salt-asparagine medium were placed under a 100-watt light bulb at a distance of 2 ft. Five additional cultures were protected from the light by a heavy black cloth. After 7 days, the antibiotic activity of the cultures was assayed by reducing the pH of the medium to 4.0 and autoclaving at 15 lb. pressure for 15 minutes. One ml of the suspension of dead bacterial cells in the culture medium was diluted with 9 ml of potato dextrose agar in petri dishes. The plates were inoculated with *Rhizoctonia* sp. and the radius of the colonies recorded after 24 hours. No significant difference was noted in lateral growth between cultures grown in the dark and cultures grown in the light. Average colony radius of the fungus on medium containing bacteria grown in the light was 2.8 mm, whereas the average radius of the fungus colonies on medium containing bacteria grown in the dark was 2.9 mm. Both sets of control plates averaged 6 mm. It was concluded that the antibiotic produced by isolate No. 65 differs from subtilin in that it is not light sensitive.

To determine the best extraction process, extractions were made from dried bacterial powder with ethanol by a method described by Jansen and Hirschmann (7) involving pH changes. A red precipitate was obtained, which was completely inactive as an antibiotic in inhibiting the growth of *Rhizoctonia* sp. Extractions from as much as 1 g of bacterial powder failed to inhibit the growth of the fungus when added to 10 ml of nutrient agar in a single petri dish. Extractions were made from 0.25 g quantities of bacterial powder with ethanol, methanol, and n-butanol. The alcohols were distilled off and the residue assayed for antibiotic activity against *Rhizoctonia* sp. The n-butanol extraction completely inhibited growth of the fungus whereas the ethanol extraction showed slight and the methanol extraction very slight inhibition. Babad et al (2) have described a method of extracting antibiotic from a liquid culture medium with n-butanol. When this method was employed, slightly higher yields of antibiotic were obtained from a 4-day old culture of *B. subtilis* than were obtained from dried bacterial powder taken from an equal volume of the same culture.

Hirschhorn et al (5) reported that carbohydrates interfered with the production of subtenolin, another of the antibiotics produced by *B. subtilis*. To determine the effect of carbohydrates on the ability of isolate No. 65 to produce antibiotic, a number of media were compared. Potato dextrose broth, salt-asparagine medium, beet molasses medium (8), and a carbohydrate-free medium described by Hirschhorn et al (5) were each prepared in quintuplicate in 200 ml quantities in 1 liter flasks. One hundred ml of medium from each flask was assayed for antibiotic activity 2 days after inoculation and again after 4 days. The pH of the culture medium was reduced to 4.0 before autoclaving. Triplicate plates containing 3 ml of potato dextrose agar were used to assay the antibiotic activity in each medium. The plates were inoculated with *Rhizoctonia* sp. and the lateral growth of the fungus recorded after 48 hours. The 100 ml of culture medium remaining in the flasks was utilized to repeat this procedure 4 days after inoculation.

Enough antibiotic had been produced in the potato dextrose, salt-asparagine and beet molasses media in 2 days to inhibit completely the growth of *Rhizoctonia* sp. Average colony radius on the medium containing the antibiotic produced in 2 days in the carbohydrate-free medium was 7.9 mm as compared to 11.5 mm on the control plates. After 4 days, the antibiotic produced in the carbohydrate-free medium reduced the average colony radius to 5.9 mm. It was concluded that the production of antibiotic by isolate No. 65 was stimulated by the presence of carbohydrate in the medium and retarded when carbohydrate was absent.

In greenhouse studies, it was found that the amount of manure in the soil affected the ability of *B. subtilis* to control damping-off of sugar beet caused by *Rhizoctonia* sp. To study further this relationship in culture, various proportions of pulverized manure were added to water agar. The medium was autoclaved and the production of antibiotic on it assayed by means of the bacterial streak method. The concentrations of manure used were 0, 1, 10 and 100 g/l. The control plates were not streaked with bacteria. There was no production of antibiotic by *B. subtilis* on water agar containing no manure. Inhibition of the fungus on the preparations containing 1 and 10 g/l of manure was evident 4 days after inoculation; however, it was evident after only 2 days on the medium containing 100 g/l of manure. It may be concluded from this study that manure contains all the substances *B. subtilis* requires to inhibit the growth of *Rhizoctonia* sp.

Disease control studies.—To determine whether there were any injurious effects on plant development from inoculating the soil with heavy suspensions of *B. subtilis*, the following test was conducted. Sterile and non-sterile soil in pots was inoculated with 30 ml of a suspension of *B. subtilis* and the soil planted to sugar beet. There were no significant differences between initial stands of plants when compared with plants grown in noninoculated soil. Likewise, there were no obvious differences between the roots and tops of plants grown in inoculated soil for 6 weeks when compared with control plants.

B. subtilis and *Rhizoctonia* sp. were cultured together for different periods of time to determine what effect the bacterium might have on the ability of the fungus to parasitize sugar beet seedlings. The fungus was cultured on boiled corn seed for 1 week. The corn seed and fungus were then covered with sterile distilled water and the culture inoculated with *B. subtilis*. The bacteria and fungus were cultured together for periods of 48 hours, 1 and 2 weeks. Afterwards, the corn seed and fungus were washed in tap water and the fungus

used to inoculate 5 pots of sterile soil. Five additional pots of sterile soil were inoculated with *Rhizoctonia* sp. that had not been in contact with bacteria. Five pots of sterile soil were included as controls.

The first and second week cultures produced final stands of 52 and 48 per cent, respectively. Cultures of the fungus that contained no bacteria reduced the final stand to 25 per cent, whereas the 48-hour cultures gave a slightly higher increase in final stand over this figure. The test was repeated twice with essentially the same results. It was concluded that isolate No. 65 of *B. subtilis* had the ability to inhibit *Rhizoctonia* sp. when the fungus and bacterium were cultured together for periods as short as 48 hours.

Sterile soil contained in 10 5-in. clay pots was inoculated with *B. subtilis* to determine to what extent the bacteria could persist in the soil after inoculation. Five pots were maintained at a temperature of 15°C and the other 5 at 30°C. Soil samples were taken from each pot 7 and 9 days after inoculation. Dilution plates prepared from samples of freshly inoculated soil showed the bacterial population to be extremely variable; however, after 7 days the bacterial population appeared to be more stable. Nine days after inoculation the average bacterial population of the samples taken from the pots at 15°C was 25,000 and at 30°C it was 47,000. Because of the better distribution of *B. subtilis* in inoculated soil after 7 days, it seemed advisable to pre-inoculate the soil with bacteria in future studies of the inhibition of *Rhizoctonia* sp. by *B. subtilis*.

To study further the antagonistic relationship, 5 pots of sterile soil were inoculated with *B. subtilis*. Seven days later, the pots were inoculated with *Rhizoctonia* sp. Five additional pots were inoculated with the fungus alone and 5 were used as controls. After the sugar beet seed had been sown, the pots were placed in a temperature chamber at 27°C. At this temperature, the seed germinated promptly, giving a comparatively high initial stand. Soil inoculated with both fungus and bacteria showed 18 per cent pre-emergence damping-off compared to 37 per cent in the soil inoculated with the fungus only. The soil inoculated with both the fungus and bacterium supported a final stand of 25 per cent while the fungus inoculated soil supported a final stand of only 9 per cent. In each case, the majority of decrease in stand was attributed to a post-emergence damping-off.

The preceding experiment was repeated at 21°C and at this temperature there was a delay in germination and almost all the loss in stand was attributed to pre-emergence damping-off. *Rhizoctonia* sp. proved to be less pathogenic at 21°C than at 27°C (Fig. 1). The final stand supported by the fungus inoculated soil was 27 per cent. Inoculation of the soil with both the fungus and the bacterium resulted in a final stand of 65 per cent. This represents an increase in stand of 38 per cent over the fungus inoculated soil. It was thus concluded that inhibition of *Rhizoctonia* sp. by *B. subtilis* was greater at the lower temperature.

To determine what effect manure might have upon the antagonistic relationship between *B. subtilis* and *Rhizoctonia* sp. in the soil, a number of soil-manure mixtures were prepared. Marshall silty-clay loam containing 0, 25, 50, and 75 per cent well rotted manure, by volume, constituted the soil-manure mixtures used in this test. They were sterilized in 5-in. pots and quintuplicate sets of each were inoculated

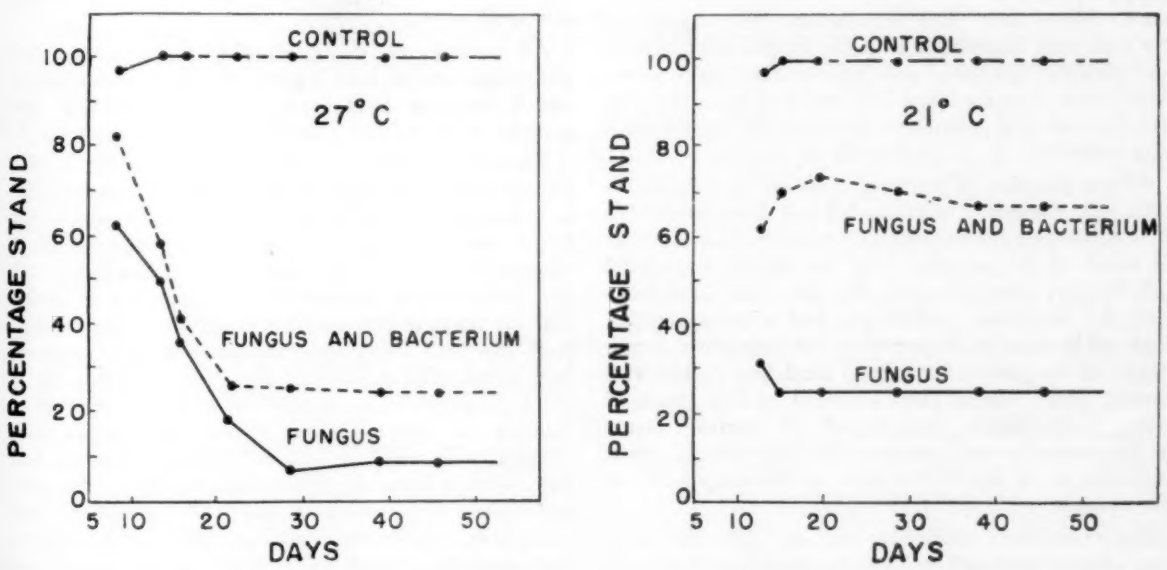


FIG. 1. Percentage stand of sugar beet seedlings at 27° and 21°C on sterile soil inoculated with *Rhizoctonia* sp. alone and with *B. subtilis* as compared to that on noninoculated soil.

with *B. subtilis*. After 7 days, each of the mixtures was inoculated with *Rhizoctonia* sp. At this time, an additional 5 pots of each of the various soil-manure mixtures and plain soil, previously sterilized, were inoculated with *Rhizoctonia* sp.

The most serious losses due to damping-off in this test were found in the mixture containing 75 per cent manure. The fungus inoculated mixture supported a final stand of 10 per cent compared with a 43 per cent stand in the fungus and bacterial inoculated mixture. The least damping-off occurred in the soil containing no manure. Here the fungus inoculated soil produced a stand of 73 per cent whereas the fungus and bacterial inoculated mixture produced a stand of 83 per cent. Maximum benefit from inoculation of the soil with *B. subtilis* was obtained in the 25 per cent manure mixture which showed an increase in stand of 43 per cent due to the introduction of *B. subtilis* into the mixture. The 75 and 50 per cent mixtures showed comparable increases in stand of 33 and 30 per cent respectively. Minimum benefit derived from the inoculation of the mixtures with bacteria was recorded in soil containing no manure where an increase in stand of 10 per cent was observed. Although the addition of manure amendments to soil inoculated with the *Rhizoctonia* increased the amount of damping-off of the seedlings, the addition of *B. subtilis* to the soil increased final stands considerably in all cases.

The following experiment was conducted to determine whether *B. subtilis* was capable of inhibiting *Rhizoctonia* sp. in non-sterile soil and to determine, if possible, some of the factors influencing inhibition. Three groups of 5 pots each, containing coarse sand, nonamended soil and amended soil (greenhouse soil) were inoculated with *B. subtilis*. The amended soil was composed of 3 parts soil, 1 part manure, and 1 part sand. Seven days after inoculation, the contents of the pots were inoculated with the fungus only. Three noninoculated groups of pots were retained as controls. After sugar beet seed had been sown in pots, the pots were placed in a greenhouse in which the temperature was controlled at approximately 21°C.

Severe damping-off occurred with all the treatments (Fig. 2). However, nonamended soil inoculated with *B. subtilis* and *Rhizoctonia* sp. supported an increase in stand of 60 per cent over the fungus inoculated soil 30 days after planting. Amended soil inoculated with the bacterium and fungus had a corresponding increase in stand of 80 per cent. Pre-emergence damping-off in the fungus inoculated sand was particularly severe. Not a single plant emerged in this group of pots. Considerable damping-off of control plants in the amended and nonamended soils proved, upon isolation, to be due to the same or similar species of *Rhizoctonia*.

An experiment similar to the one just described was conducted simultaneously to determine the effect of a high nitrogen nutrient solution upon the inhibition of *Rhizoctonia* sp. by *B. subtilis*. Plants were watered every other day with Hoagland's "solution 1" (6) containing twice the specified amount of potassium nitrate.

Considerable increases in stand on bacterial and fungus inoculated soil and sand in the 3 groups of pots were observed as a result of watering the plants with high nitrogen nutrient solution (Fig. 3). In the fungus inoculated soils, increases in stand of 80 and 90 per cent were recorded for the nonamended and amended soils respectively. The fungus and bacterial inoculated soils showed increases in stand of 38 and 83 per cent on the nonamended and amended soils respectively. Since the *Rhizoctonia* was indigenous in both the nonamended and amended soils, one would expect corresponding increases in stand of the control plants in both soils due to the addition of nutrient solution to the soils. This is borne out in the data by increases in stand of 41 and 80 per cent for the respective soils. An increase of 67 per cent in stand was recorded for the fungus and bacterial inoculated sand. At the completion of the experiment, roots of plants receiving nutrient solution were compared with those of plants grown in comparable soil or sand which had none. No difference in root development was recorded except in sand where the nutrient solution definitely benefited root development in comparison with plants grown in sand that had not received nutrient solution.

From the experiments with non-sterile soil and sand, it was concluded that limited control of damping-off of sugar beet caused by *Rhizoctonia* sp. could be obtained in soil inoculated with *B. subtilis*. Soil amended with both manure and sand appeared to favor control, especially when nutrient solution high in nitrogen was added to the soil. It is questionable whether the sand in the amended soil aided in the control since damping-off was most severe when sand was used alone.

All greenhouse experiments with non-sterile soil were repeated at least twice. Although variation in stands occurred from experiment to experiment, the general relationships discussed remained unchanged.

Discussion.—The antibiotic produced by isolate No. 65 has 3 characteristics in common with subtilin. It is largely retained by the bacterial cells and is not found free in great quantity in the medium. It is associated with a dark blue color characteristic of the formation of chelates in the presence of ferric ions. It tends to lose activity as pH 7.0 is approached from the acid side. This antibiotic differs, however, in a number of important ways from subtilin. It is not light sensitive as is subtilin, and the most active fraction of the antibiotic is not extractable with ethanol, as is the case with subtilin, but it is extractable with n-butanol. Other than subtilin, the only antibiotics with which it might be compared are bacitracin, toximycin, and an unnamed antibiotic described by Babad et al (2), all of which are extractable with n-butanol. Both toximycin and the antibiotic described by Babad have strong antifungal

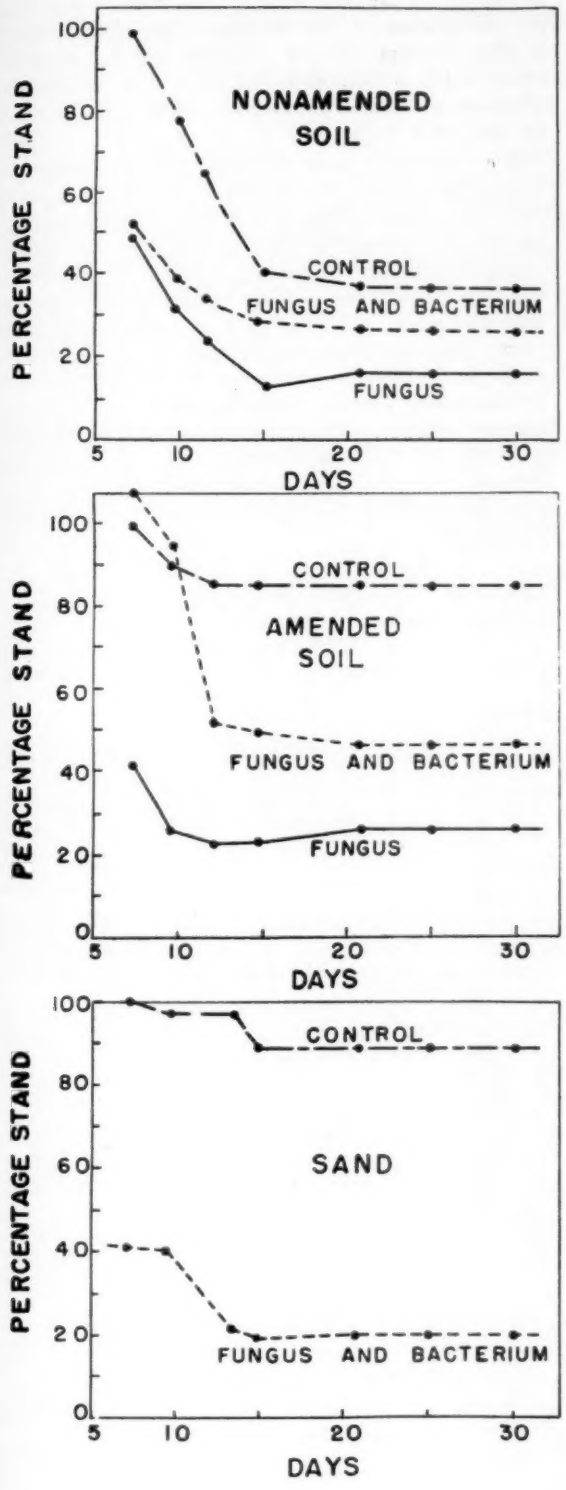


FIG. 2. Percentage stand of sugar beet seedlings on nonsterile soil and sand inoculated with *Rhizoctonia* sp. alone and with *B. subtilis*. Control plants were grown in noninoculated soil.

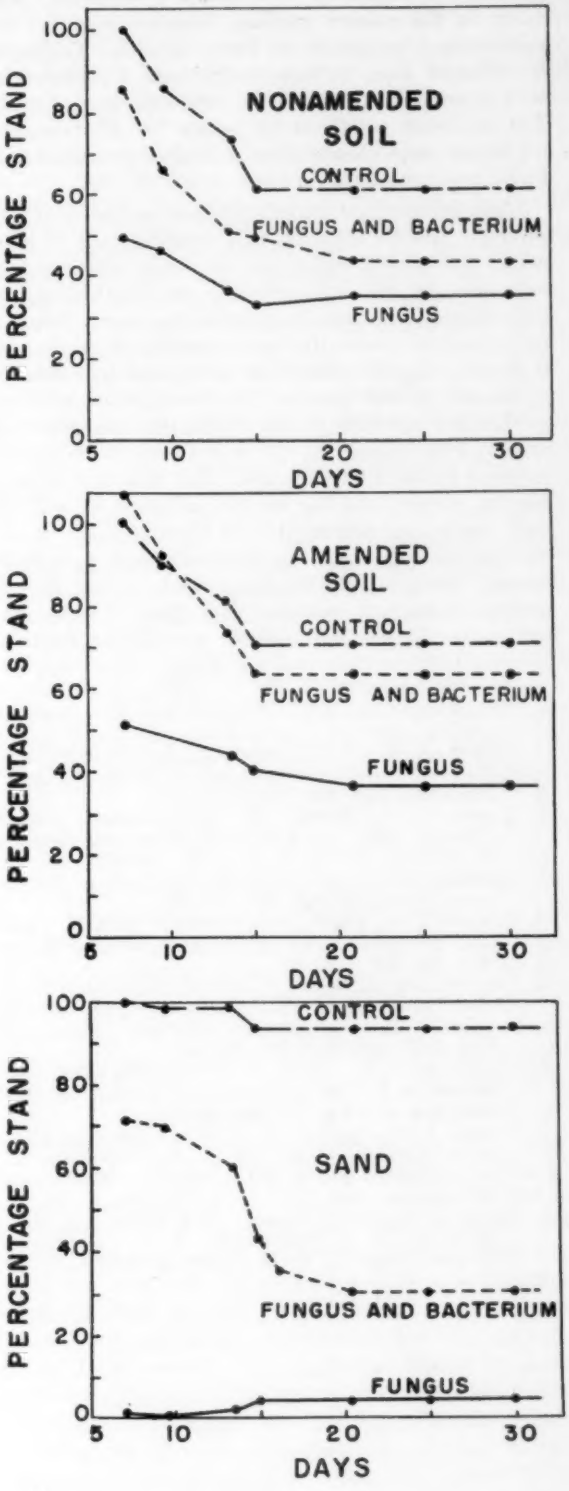


FIG. 3. Percentage stand of sugar beet seedlings on nonsterile soil and sand inoculated with *Rhizoctonia* sp. alone and with *B. subtilis*. All plants were watered with a high nitrogen nutrient solution.

properties. Bacitracin and Babad's antibiotic both occur in the culture medium, however, and are not concentrated within or on bacterial cells. Toximycin is obtained from culture centrifugate by extraction with n-butanol (13) as is the antibiotic in this study. The antibiotic produced by isolate No. 65 resembles toximycin more closely than any other described antibiotic produced by *B. subtilis*.

Some antagonistic microorganisms secrete metabolic products into the substrate that inhibit growth of other microorganisms growing on the same substrate. In soil, much of the antibiotic may be adsorbed on soil colloids and inactivated. Subtilin has been shown to be deposited externally on bacterial cell walls (7). It is only slightly soluble in water and its solubility is lowered in the presence of electrolytes. Although no data are available on the solubility of the antibiotic produced by isolate No. 65, it is known to be largely retained by the bacterial cells. This fact may account for the greater stability of the antibiotic in soil. In both sterile and non-sterile soil this antibiotic retains its activity longer than an antibiotic such as streptomycin, which is rapidly inactivated in soil due to adsorption on soil colloids (12). Since 7 days after inoculation of the soil with *B. subtilis* the bacterial population of the soil is fairly uniform, it follows that the distribution of the antibiotic throughout the soil is also uniform. If the solubility of the antibiotic under study is low, as is the case with subtilin, this situation will allow for a more even distribution of the antibiotic in the soil.

The limited control of damping-off achieved in non-sterile soil may be explained by the presence of other organisms competing with *B. subtilis* for nutrients and by their rendering the soil microenvironment less habitable through various other processes. The addition of high nitrogen nutrient solution to the soil may favor the growth of *B. subtilis* in preference to other forms, thus enabling the bacteria to produce more antibiotic. Since no difference in root development was observed between plants grown in soil receiving nutrient solution and those not receiving it, this appears to be the most plausible explanation. The possibility remains, however, that plant resistance may have been altered due to the addition of the nutrient solution to the soil.

Department of Plant Pathology
University of Nebraska
Lincoln 1, Nebraska

LITERATURE CITED

1. Anwar, A. A. 1948. Factors affecting the survival of Helminthosporium sativum and Fusarium lini in soil. Phytopathology **39**: 1005-1019.
2. Babad, J., A. Pinsky, R. Turner-Graff, and N. Sharon. 1952. An antifungal polypeptide produced by Bacillus subtilis. Nature **170**: 618-619.
3. Bamberg, R. H. 1931. Bacteria antibiotic to Ustilago zeae. Phytopathology **21**: 881-890.
4. Cordon, T. C., and C. M. Haenseler. 1939. A bacterium antagonistic to Rhizoctonia solani. Soil Sci. **47**: 207-214.
5. Hirschhorn, H. N., M. A. Bucca, and J. D. Thayer. 1948. Subtenolin. An antibiotic from Bacillus subtilis. I. Bacteriologic properties. Proc. Soc. Exp. Biol. and Med. **67**: 429-432.
6. Hoagland, D. R., and D. I. Arnon. 1950. The water-culture method for growing plants without soil. Cal. Agr. Exp. Sta. Cir. 347 Rev. ed.
7. Jansen, E. F., and D. J. Hirschmann. 1944. Subtilin—an antibacterial product of Bacillus subtilis, culturing conditions and properties. Archiv. Biochem. **4**: 297-309.
8. Lewis, J. C., R. E. Feeney, A. J. Garibaldi, H. D. Michener, D. J. Hirschmann, H. D. Traufler, A. F. Langlykke, H. D. Lightbody, J. J. Stubbs, and H. Humfeld. 1947. Subtilin production in surface cultures. Archiv. Biochem. **14**: 415-425.
9. Porter, C. L. 1924. Concerning the characters of certain fungi as exhibited by their growth in the presence of other fungi. Am. Jour. Bot. **11**: 168-188.
10. Sanford, G. B. 1926. Some factors affecting the pathogenicity of Actinomyces scabies. Phytopathology **16**: 525-527.
11. Sanford, G. B., and W. C. Broadfoot. 1931. Studies of the effect of other soil inhabiting microorganisms on the virulence of Ophiobolus graminis. Sci. Agr. **11**: 512-528.
12. Siminoff, P., and D. Gottleib. 1951. The production and role of antibiotics in the soil: I. The fate of streptomycin. Phytopathology **41**: 420-430.
13. Stessel, G. J., C. Leben, and G. W. Keitt. 1953. Partial purification and properties of the antifungal antibiotic toximycin. Phytopathology **43**: 23-26.
14. Vasudeva, R. S. 1952. Investigations of the inhibitory action of Bacillus subtilis on Fusarium udum Butl., the fungus causing wilt of pigeon-pea. Ann. Appl. Biol. **49**: 229-238.
15. Waksman, S. A. 1947. Microbial Antagonisms and Antibiotic Substances. The Commonwealth Fund. New York, N. Y.

THE ROLE OF A SELF-INHIBITOR IN THE GERMINATION OF RUST UREDOSPORES[1]

Paul J. Allen

SUMMARY

The rate of germination of uredospores of *Puccinia graminis* f. sp. *tritici* is inversely related to the quantity of spores present under given conditions. An actively metabolizing population of spores can bring about inhibition in other spores separated from them by an air gap. Self-inhibition is reduced, but not abolished by aeration.

By floating spores en masse under aerobic conditions for 8–24 hours, they are so altered that they no longer show any of the above signs of self-inhibition. These changes do not occur anaerobically. The solutions on which uredospores have been floated for a few hours or more contain a substance or substances highly active in preventing the germination of other uredospores. The activity of different concentrations of the aqueous solution of inhibitor parallels the curve of self-inhibition with different quantities of spores. The production of inhibitor occurs aerobically but is not appreciable under anaerobic conditions. Little or no inhibitor is produced at pH's above 7.0.

The activity of crude solutions of inhibitor is reduced by aeration at pH 6.0 but not at 7.3, is retained on heating to 100°C. if the container is sealed, and is removed by exposure to glass surfaces.

The effectiveness of inhibitor solutions against pre-floated spores is not appreciably affected by the concentration of potassium phosphate or the O_2 tension, but increases with rising pH above 6.0 and decreases with increasing CO_2 tension up to at least 2.5 per cent. Most of the differences in the effects of external variables tested on the germination of untreated and pre-floated spores can be accounted for by the effects of these variables upon the production or effectiveness of inhibitor in the untreated spores.

Germination of untreated uredospores is stimulated by dinitrophenol, methyl naphthoquinone, and coumarin, the optimal concentration increasing with increasing quantity of spores. The germination of pre-floated spores is much less sensitive to these compounds.

The activity of inhibitor solutions in preventing germination is also overcome by dinitrophenol, methyl naphthoquinone, and coumarin. Evidence is presented that the action of dinitrophenol does not depend upon formation of a dinitrophenol-inhibitor complex in the medium.

Vagaries in uredospore germination are largely explicable in terms of the germination inhibitor found in aqueous solutions. They can be eliminated by an appropriate pre-floating treatment, and spores can thus be obtained which will germinate in large quantities on a consistent and predictable schedule.

Interest in the germination of rust uredospores has arisen repeatedly from efforts to analyze factors controlling the development of plant diseases. Unlike the spores of many saprophytic fungi, rust uredospores germinate erratically and inconsistently (1, 3, 6, 7, 10, 15, 18, 22, 24). Standardization of the conditions of host culture (16, 24), of the age of pustule from which spores were collected (15, 17), and of the period of storage prior to germination tests, although contributing to greater reproducibility, have proven insufficient to eliminate irregularities in germination, which seem to depend in part upon uncontrolled factors operating during the germination tests (3, 9, 12, 17). The present investigation[2] arose from attempts to establish the nature of these factors and to eliminate them as sources of variability.

Germination was found to be remarkably sensitive to almost any kind of modification in the test conditions. The shape of the container and the volume of solution affected the rate of germination, which was slower in Erlenmeyer flasks than in beakers and slower on small volumes of liquid than on large volumes. Sealing the test vessels consistently retarded germination. An experiment to determine the relation of air volume to germination, with equal volumes of liquid in each of a series of simliar flasks in which the air volume was varied by addition of glass beads, indicated clearly that the rate of germination was proportional to the volume of glass beads. Of the many factors examined, it soon appeared, however, that the most important was the number of spores in the test vessel; the larger the number, the more slowly the spores germinated, and with very large numbers there was no germination at all. This fact suggested that a germination inhibitor might be responsible for the erratic behavior of these spores. Studies following from this hypothesis have established the participation of an inhibitor in uredospore germination, and have defined some of the major factors influencing its production and activity. Since adverse effects of large numbers of spores upon germination have been noted with saprophytic fungi also (8, 9), germination inhibitors may be of more widespread importance in the regulation of development of fungus spores than heretofore recognized.

METHODS OF STUDYING SPORE GERMINATION.—Uredospores of *Puccinia graminis* Pers. f. sp. *tritici* Erikss. &

[1] Accepted for publication December 21, 1954.
[2] The assistance of H. P. Lankelma, Jr. in the early stages of this study is gratefully acknowledged.

E. Henn., Race 56, were harvested by means of a cyclone collector (23) and stored at 6°C in a desiccator over saturated squeous NaBr, at a humidity of approximately 60 per cent. One mg of these spores (about 15 per cent moisture) contained approximately 3×10^5 spores and was just sufficient to form a uniform film over the surface of 1.5 cm^2 of liquid. Quantities of this order are referred to as spores "en masse", whereas 1×10^3 to 3×10^3 spores on a similar area of liquid are referred to as "traces". Samples were placed on the liquid and dispersed over the surface by vibrations of an inoculating needle. When only a few spores were used, they quickly re-assembled on a small area at the bottom of the meniscus.

Germination tests were conducted in van Tieghem vessels prepared by sealing a 1.2 cm section of pyrex glass tubing to a microscope slide with sealing wax. The vessels, capacity 1.8 ml, were nearly filled with 1.6 ml of liquid and left open, or partially filled with 0.8 ml and sealed with a coverslip and vaseline. Spores were usually floated on this liquid, but in a few instances were placed on a hanging drop suspended from the coverslip above it. For experiments involving replacement of air with other gases, van Tieghem vessels equipped with 2 sidearms and stopcocks were used. These vessels had a capacity of 5.0 ml, and received 1.2–2.0 ml of liquid. Air was replaced by flushing with ½–1 liter of the test gas over a period of 3–4 minutes and the chamber was then sealed, or slow aeration was continued until the experiment was concluded.

The criterion for germination was emergence of a germ tube to a length exceeding its width. The percentage germination was obtained from a count of 50–150 spores, usually 100.

Solutions were buffered with potassium phosphate buffer to give a final concentration of 0.01M at pH 6.0 and tests were conducted in light at 20–23°C, close to the reported optimum (14, 17, 22). Under these conditions of temperature and buffering, no significant change in pH occurred during germination. For studies on the effect of pH, the ratio of $KH_2PO_4/K_2H\ PO_4$ was varied and the final pH checked by measurement with a Beckman meter.

Results.—*Effects of the size of spore population upon germination.*—When 1×10^3 to 3×10^3 uredospores were floated on dilute phosphate buffer in open van Tieghem vessels within a few days after harvesting, germination began about an hour after contact with the solution and continued almost linearly with time until most of the spores had germinated (Fig. 1, A). Under similar conditions, spores that had been in storage for longer periods germinated more slowly and showed a more pronounced delay following emergence of the first few germ tubes (Fig. 1, B). After prolonged storage, complete germination in these small populations required a day or more. If 3×10^5 spores (1 mg) or more were floated under the same conditions, little or no germination occurred at any time, regardless of the previous period of storage. With intermediate quantities of spores, the amount of germination at a specified time was inversely related to the spore concentration (Fig. 2, A). At each spore concentration, germination was considerably retarded by sealing the van Tieghem vessel (Fig. 2, B). On the other hand, continuous aeration of the vessels accelerated germination. The effects of both sealing and aerating were most marked with small populations of uredospores.

These results indicated that germination was adversely affected by conditions produced by the spores themselves, and that unfavorable conditions developed most markedly in large populations and in a stagnant atmosphere. To determine whether the suppression of germination was reversible, 1.5 mg of spores were floated in a van Tieghem vessel, the vessel was sealed to ensure sustained inhibition, and samples of approx-

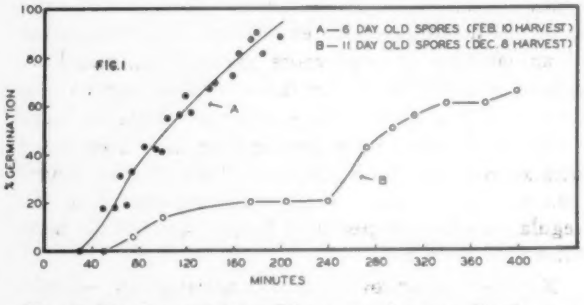


Fig. 1. Total germination of untreated spores floating on 1.6 ml 0.01 M phosphate buffer in open vessels.

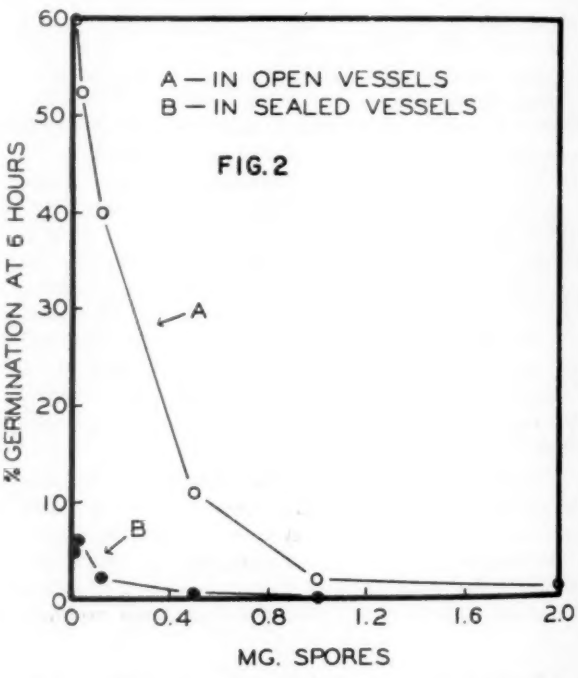


Fig. 2. Effect of spore concentration on germination of spores more than 1 month old, floated on 1.0 ml liquid in van Tieghem vessels.

imately 1×10^3 spores were removed at intervals. Each sample was washed by floating for a few seconds on distilled water and was immediately transferred to fresh 0.01 M phosphate buffer, pH 6.0, in an open van Tieghem vessel. Although there was no germination in the original vessel in 23 hours, nearly 100 per cent germination had occurred in all samples transferred to fresh solutions, regardless of the previous period of suppression. Between successive samples, however, marked changes appeared in the rate of germination (Fig. 3). Whereas spores that were removed without a prior period of floating (referred to hereafter as "untreated" spores) germinated slowly and irregularly, those that were first floated en masse ("pre-floated" spores) germinated more and more rapidly and regularly as the period of pre-floating increased. After 6 or more hours pre-floating, the time-germination curve was almost a perfect sigmoid curve, characteristic of the unimpeded germination of a normal population. Furthermore, spores removed after these longer periods of pre-floating germinated equally rapidly in sealed or in open vessels, in traces or en masse. When wetted by mechanical agitation they germinated beneath the liquid surface as rapidly as floating spores.

None of the foregoing changes occurred if pre-floating was carried out under anaerobic conditions, but several hours of anaerobiosis did not impair germinability and did not interfere with the effectiveness of a subsequent period of aerobic floating. Aerobic metabolism is therefore concerned in effecting the changes that produce a population of spores that will germinate without evidence of self-inhibition. The expression of these changes, however, does not occur until the spores are removed from the pre-floating medium, and must therefore be reversibly prevented by substances accumulating in this medium during the pre-floating treatment.

Inhibition by substances released from uredospores.—The occurrence of an inhibitor in the aqueous phase was demonstrated by removing the solution on which a mass of spores had floated for several hours and testing its influence upon the germination of a trace of spores. The solutions, produced by floating 1–5 mg of spores/ml 0.01M phosphate at pH 6.0 in a sealed vessel, were removed by inserting a pipette beneath the floating film of uredospores and transferring a sample of the spore-free solution to another vessel. On this solution, which will be referred to as a crude solution of inhibitor, germination was completely prevented in both untreated and pre-floated spores. When the inhibitory solution was diluted with phosphate buffer, the dilution-activity curves obtained showed that considerable inhibition still occurred at a 10 × dilution (Fig. 4). The action of these preparations against pre-floated spores was more regular and, because of the better germination in controls, was more pronounced than with untreated spores. Pre-floated spores were therefore used for most of the subsequent experiments with solutions of inhibitor.

An estimate of the maximum possible concentration of the active substance was obtained by measurements of loss in dry weight and CO_2 production. (Evidence that active substances is not CO_2 is presented below). During the first 6 hours after contact with water, the total loss of spore material was found to be less than 10 per cent, of which approximately ¼ was accounted for as CO_2. In a 10 × dilution of 1.0 ml of solution, which shows considerable inhibitory action (Fig. 4), the concentration of all metabolites other than CO_2 originating from 1.0 mg of spores cannot therefore exceed 7.5 ppm, only a part of which may be the active material. Similar calculations, based on the fact that 0.005 mg of spores was sufficient to reduce germination in a sealed vessel to a few per cent (Fig. 2), gave 0.5 ppm for the concentration of metabolites in an inhibitor solution.

Some properties of the inhibitor.—Most of the

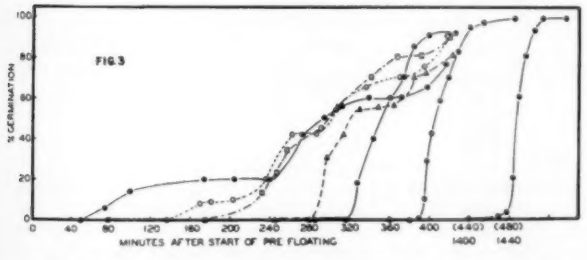


FIG. 3. Germination following transfer of a few uredospores to fresh solutions from a large population. The lower figures on the abscissa refer only to the curve on the extreme right. See text for details.

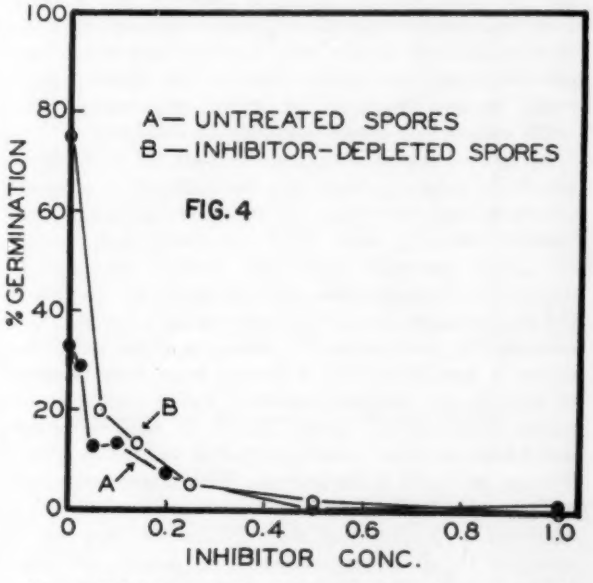


FIG. 4. Inhibition by liquid on which spores were previously floated. The concentration of crude inhibitor is shown as fractions of the original preparation. Tests carried out in sealed vessels. A) Test spores 1 month old. B) Test spores 5 days old, pre-floated for 20 hours.

TABLE 1.—*Effect of aeration and of heating on the activity of crude inhibitor solutions*[a]

Exp. No.	Treatment	Concentration giving approximately ½ maximal germination[b]		Amount of activity lost by treatment
		Before treatment	After treatment	
1 a	aerate at pH 5.7 for 85 min. with air, test at pH 6.7	<0.04	0.16	over ¾
1 b	aerate at pH 7.3 for 85 min. with air, test at pH 6.7	<0.08	<0.08	none
2 a	aerate 80 min. with N_2	<0.06	0.50	nearly all
2 b	aerate 80 min. with air	<0.06	0.16	about ½
3 a	100°C for 30 min.—open	<0.12	>0.50	nearly all
3 b	100°C for 30 min.—sealed	0.25	<0.12	none (increase?)

[a] Concentration of inhibitor solution based on the original inhibitor as 1.0. Both aeration and assay were carried out at pH 6.0 except as otherwise indicated.
[b] i.e., ½ the germination occurring in complete absence of inhibitor.

activity of a crude solution of inhibitor was lost after aeration at or below pH 6.0 for 1–2 hours, but the loss was negligible at pH 7.3 (Table 1, Experiments 1a and 1b). Bubbling N_2 instead of air through a solution at pH 6.0 also reduced its activity (Table 1, Experiments 2a and 2b). Heating to 100°C removed inhibitor activity if the heating was carried out in an open tube, but not if the tube was sealed (Table 1, Experiments 3a and 3b). These experiments indicate that the loss of inhibitor upon aeration was not a result of oxidation but of volatilization, and suggest that the active principle is a heat stable substance with weakly acidic properties.

The activity of crude inhibitor solutions was removed by glass surfaces. Samples of a solution were transferred to van Tieghem vessels containing different quantities of glass beads of about 0.2 mm diameter. After 3 hours the solutions were removed, diluted 2×, and tested for activity (Table 2). Forty-eight cm^2 of glass surface removed most of the activity from this preparation of inhibitor. Although the surface contributed by the van Tieghem test vessel was not enough to have much effect in this experiment, it could become a factor of major importance with small volumes or dilute solutions of inhibitor.

In view of the evidence that self-inhibition involved a volatile substance and that the activity of aqueous solutions was volatilized at room temperature, preliminary attempts were made to distill and recover an active material. Although limited attempts to date have been unsuccessful, the methods employed did not preclude loss of the material by adsorption on glass during distillation. Transmission of the inhibition across a gas phase has, however, been demonstrated in sealed van Tieghem vessels. The germination of traces of untreated spores placed on hanging drops was inhibited if the vessels contained additional spores floating on liquid in the bottom. With larger quantities of spores in the bottom of the vessel, greater inhibition occurred in the hanging drop. Since the number of spores in the drop was similar for all vessels, the inhibition must be attributed to substances passing across an air gap from the spores below. It cannot be ascribed either to reduced O_2 tension or increased CO_2 tension (see Fig. 7 A and 8 A). When dry spores were placed in the bottom of a vessel without any liquid, there was no inhibition of test spores in a hanging drop above.

Conditions affecting inhibitor production.—Some assays have been made of the amount of inhibitor activity arising under different conditions of incubation of untreated spores. Although such assays may not be quantitatively precise owing to the sequestering of inhibitor by the glass vessels used, they are sufficiently accurate, when the inhibition of germination of pre-floated spores is used as an index, to show any large quantitative differences. Assays were made by measuring the amount of germination occurring on successive dilutions of the liquid on which a standard quantity of spores had previously been incubated. Estimates of the relative amounts of inhibitor activity were then obtained from the dilutions required to give similar but incomplete inhibition of the test spores.

Very little inhibitor accumulated under anaerobic conditions. Under aerobic conditions, no significant difference was found between inhibitor production on distilled water and on 0.01 M phosphate buffer, but marked effects of the pH of the phosphate were detected. Incubation at pH 5.5–6.0 gave solutions with high activity when tested at pH 6.4, whereas solutions obtained by incubation at pH 7.0 and likewise tested at pH 6.4 were almost completely inactive. The low activity was not due to inactivation at the

TABLE 2.—*Removal by glass surfaces of the inhibitor activity of a crude inhibitor solution*[a]

gm. of glass beads	area of absorbing surface, $cm.^2$	% germination
none	4	2
0.05	9	20
0.10	15	20
0.20	26	38
0.40	48	60
Control without inhibitor	—	95

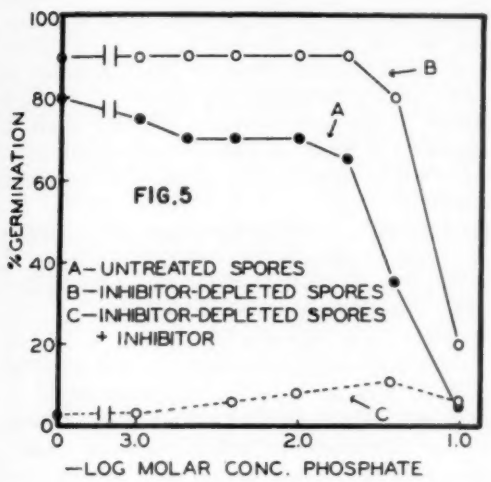
[a] Figures for area of glass surfaces include the surface of the slide and well in contact with the solution. After 3 hours in contact with the glass beads, solutions were removed, diluted 2× and their effects on the germination of pre-floated spores determined at 60 minutes.

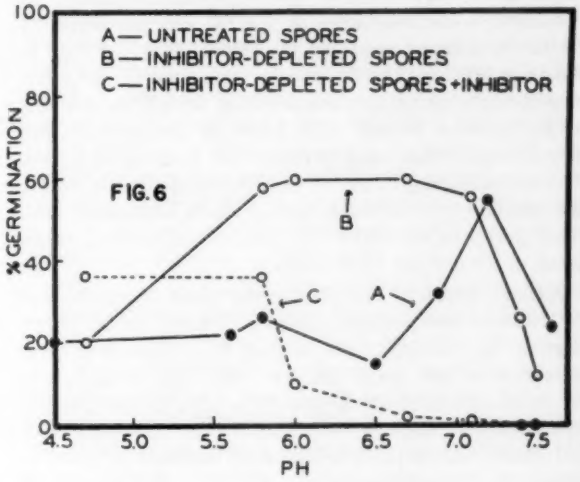
higher pH (see Table 1 and Fig. 6, C). Since spores en masse did not germinate even at alkaline reactions, some inhibitor was probably produced even above pH 7.0.

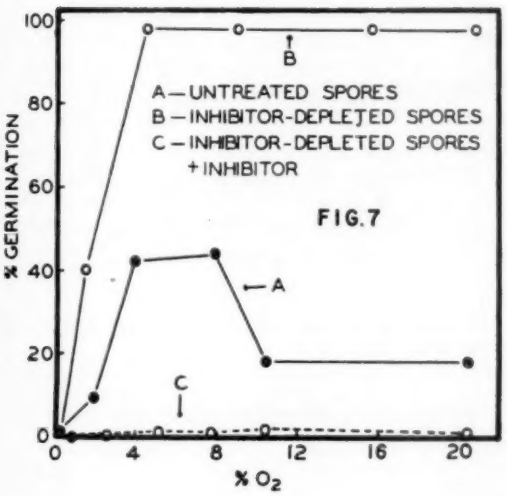
Influence of some external variables on germination and on the activity of solutions of inhibitor.—The influence of phosphate concentration, pH, and 0_2 and CO_2 tension on the germination of untreated spores is shown in Fig. 5–8, curve A. In the same figures, curve B represents the effects of these variables on the germination of pre-floated spores. Pre-floating in all cases was for 10 hours or longer on 0.8 ml 0.01 M phosphate buffer in sealed vessels, and the resulting spores are designated as inhibitor-depleted. Curve C shows the effects of phosphate, pH, O_2 and CO_2 tension upon inhibitor-depleted spores in the presence of a fixed quantity of exogenous inhibitor.

Except for a slight reduction in the germination of untreated spores by low concentrations of potassium phosphate, this compound had similar effects upon untreated and inhibitor-depleted spores (Fig. 5 A and B). There was no significant effect of phosphate upon the action of added inhibitor (Fig. 5 C).

At pH's above 6.0, the inhibitor was clearly more effective than at lower pH's (Fig. 6 C) and in accord with this, the germination of untreated spores failed







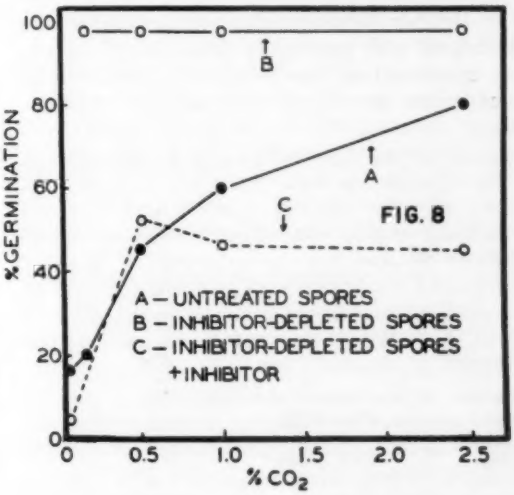


FIG. 5-8. Germination of traces of uredospores under different environmental conditions.—Fig. 5. Effect of potassium phosphate concentration, in open van Tieghem vessels. A) Spores 17 days old, germination at 90 minutes. B) Spores 29 days old, germination at 50 minutes. C) Spores 15 days old, germination at 150 minutes.—Fig. 6. Effect of pH. All spores 19 days old, germinaion in sealed van Tieghem vessels at A) 90 minutes, B and C) 60 minutes.—Fig. 7. Effect of O_2 tension, in 5 ml side-arm van Tieghem vessels aerated for 3-4 minutes with 1 liter of the test gas, then closed. A) Spores 19 days old, germination at 120 minutes. B) Spores 16 days old, germination at 80 minutes. C) Spores 38 days old, germination at 60 minutes.—Fig. 8. Effect of CO_2 tension, in 5 ml side-arm van Tieghem vessels aerated for 3-4 minutes with 1½ liters of the test gas, then closed. Above 0.5 per cent CO_2, the solutions were supplemented with $KHCO_3$ to maintain a pH of 6.0. All spores 30 days old. Germination at A) 80 minutes, B & C) 60 minutes.

to rise with increasing pH as did the germination of inhibitor-depleted spores (Fig. 6, A and B). Above neutrality, however, untreated spores produce little or no inhibitor, and therefore escaped its effects in a region where added inhibitor was most effective.

Optimal germination of untreated spores at 4–8 per cent O_2 was not connected with O_2-dependent changes in the efficacy of inhibitor (Fig. 7), but improved germination at higher CO_2 tensions, which has also been noted by other investigators (21), was correlated with a decrease in the effectiveness of exogenous inhibitor (Fig. 8). The actual level of CO_2 required to permit germination in the presence of inhibitor was not always the same, but in other experiments was found to depend upon the amount of inhibitor. Complete removal of CO_2 was not achieved by the technique used for the experiments of Fig. 8, but was at least more closely approached in other experiments by continuous aeration with CO_2-free air, or by placing 10 per cent KOH in the germination chamber and observing germination in hanging drops. The resulting depletion of CO_2 led to distinctly better germination of untreated spores when compared with their germination under the same conditions of treatment in normal air (Table 3).

Counteraction of inhibitor as the cause of stimulation by organic compounds.—Stimulation of spore germination by various compounds is a common phenomenon in the fungi (5), and has long been known to occur with rust uredospores (6, 13). In the present investigation, marked stimulation was observed with untreated spores upon addition of suitable concentrations of 2,4-dinitrophenol (DNP), 2-methyl-1, 4-naphthoquinone (MNQ), or coumarin (Table 4). The amount of DNP or MNQ required for maximal stimulation increased with increasing quantities of spores, so that a concentration that inhibited germination of a trace of spores stimulated germination in a larger population.

The nature of the DNP effect was further investigated. The concentration for optimal stimulation was found to depend upon pH as well as spore concentration. With 1 mg of spores/cm² liquid surface, the optimal concentration was approximately 0.5×10^{-5} M at pH 5.0, 1.0×10^{-5} M at pH 6.0, and 5.0×10^{-5} M at pH 7.0. The effectiveness of low concentrations of DNP was increased by aeration, and the time-germination curve of untreated spores in the presence of DNP resembled closely that of inhibitor-depleted spores in the absence of DNP. These findings indicated that DNP stimulation depended upon counteraction of the inhibitor. The effect of DNP upon the action of exogenous inhibitor was therefore determined. Inhibition was prevented by 1×10^{-5} M DNP over a wide range of inhibitor concentrations (Fig. 9). With lower concentrations of DNP, counteraction was complete only at the lower concentrations of inhibitor, and the minimal concentration of DNP required to counteract was in general directly related to inhibitor concentration. Untreated as well as inhibitor-depleted spores germinated when a suitable concentration of DNP was added to an otherwise effective concentration of inhibitor.

If the effectiveness of DNP depended upon a chemical combination with the inhibitor to form an inactive complex in the solution outside the spores, the complex should be less volatile than the inhibitor itself. The loss of inhibitor upon aerating a solution should in this event be reduced or prevented by DNP. To determine whether such a complex might be involved in the action of DNP, a solution of crude inhibitor was first tested to find the minimal concentration required to prevent germination of inhibitor-depleted spores. This concentration is designated as [I]. The concentration of DNP just sufficient to counteract this concentration of inhibitor was found to be 0.5×10^{-5} M (4 mμ mols. of DNP in 0.8 ml of inhibitor solution at concentration [I]). This concentration of DNP did not permit germination with a double concentration of inhibitor [2 I]. Under these circumstances, if aeration in the presence of DNP led to a complete loss of an initial complement of inhibitor [I], the solution after aeration should be capable of neutralizing the effects of a second addition

TABLE 3.—*Effect of CO_2 removal on germination of untreated spores*[a]

No.	Treatment	Method of removing CO_2	Percentage germination: Air	Percentage germination: CO_2-free air
1	Continuous aeration	air passed through 10% KOH	30	60
2	Continuous aeration	air passed through 10% KOH	16	35
3	No aeration	10% KOH in germination chamber	4	25
4	No aeration	10% KOH in germination chamber	12	25
5	No aeration	10% KOH in germination chamber	10	55

[a] No. 1–4, readings at 80 minutes; No. 5 at 60 minutes. Age of spores: No. 1 and 3, 33 days; No. 2, 5 days; No. 4, 7 days; No. 5, 8 days. Aeration at the rate of about 2 l/hour.

TABLE 4.—*Reversal of self-inhibition by DNP, MNQ, and coumarin*[a]

DNP—23 hours		MNQ—200 minutes		coumarin—165 minutes	
Molar Conc.	% germ.	Molar Conc.	% germ.	Molar Conc.	% germ.
0	0	0	2	0	6
5×10^{-6}	3	1.2×10^{-6}	7	2.5×10^{-5}	9
1×10^{-5}	80	2.8×10^{-6}	50	5.0×10^{-5}	45
2×10^{-5}	60	5.6×10^{-6}	25	1.0×10^{-4}	50
5×10^{-5}	30	1.1×10^{-5}	0	—	—
1×10^{-4}	10	—	—	—	—

[a] All tests carried out with 1 mg of spores floated in sealed van Tieghem vessels. pH 6.0, 0.01 M phosphate used in all tests.

of inhibitor [I]. If, on the other hand, no loss of inhibitor occurred during aeration, addition of a second lot of inhibitor would yield a solution with a total complement of inhibitor [2 I] capable of preventing germination despite the presence of the 4 mμ mols. of DNP. Partial loss during aeration should yield a solution with some DNP free to counteract part of the activity of subsequently added inhibitor. In carrying out the experiment, the solutions actually aerated contained a double concentration of both DNP and inhibitor. After aeration, they were diluted with water or with inhibitor to give the desired concentrations for the final assays. Aeration resulted in loss of only part of the inhibitor, but the loss of activity was similar whether or not DNP was present during aeration (Table 5). It is therefore unlikely that a DNP-inhibitor complex capable of preventing access of the inhibitor to the spores is formed. The DNP counteraction of the inhibitor must therefore depend upon events occurring in or on the spore cells.

Like DNP, both coumarin and 2-methyl-1,4-naphthoquinone owe their stimulating effects to a counteraction of the germination inhibitor. Each prevents the action of exogenous inhibitor upon pre-floated spores and, as would be expected from the concentrations required to stimulate germination of untreated spores, more coumarin is required than DNP or MNQ. Tests have not been made to determine whether the activity of these substances should also be sought in their physiological action upon the spores.

Discussion.—The observations and experiments reported in this paper establish the fact that the uredospores of *Puccinia graminis* f. sp. *tritici* produce at least 1 substance that can prevent uredospore germination. The substance probably is volatile at room temperature, since much of the inhibitor activity of aqueous solutions may be removed by aeration. This does not require that the pure substance have a boiling point at such low temperatures. The substance cannot be carbon dioxide, since the inhibition caused by a crude solution is never decreased by raising the concentration of inhibitor, whereas the introduction of carbon dioxide at partial pressures up to 2.5 per cent may decrease but never increases the activity of an inhibitor solution.

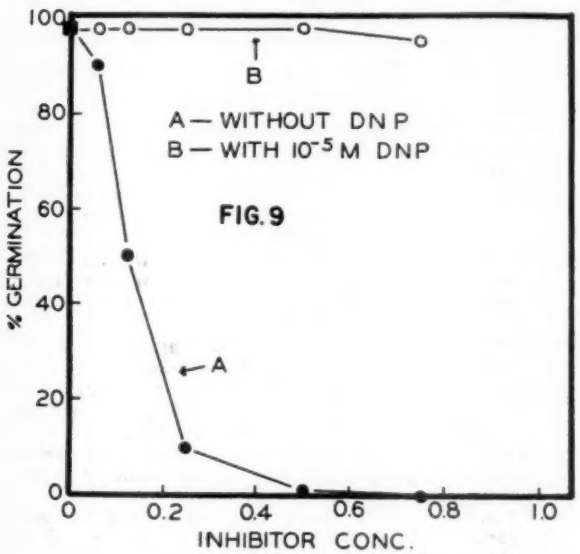


Fig. 9. Counteraction of the inhibitor by 2,4-dinitrophenol. Spores 30 days old, on 0.8 ml phosphate in sealed van Tieghems.

Table 5.—*Failure of DNP to prevent loss of inhibitor by aeration*[a]

Composition of original solution before aeration	% germination at 105 minutes: before aeration	% germination at 105 minutes: after aeration and addition of a second aliquot of I
I	5[b]	1
4 mμ mols. DNP + I	75	33
4 mμ mols. DNP + 2I	3	—

[a] See text for explanation. All treatments were carried out in 0.01 M phosphate at pH 6.0.

[b] This solution gave 30 per cent germination after aeration.

Production of the inhibitor depends upon the metabolic activity of the spores, and not simply upon the release of a pre-formed substance from the ripe spores. If it were pre-formed in the spores, it would be leached out when they are floated anaerobically or for short periods aerobically, since it can be rapidly washed out from spores which have been pre-floated aerobically for longer periods. Actually, however, it is only after prolonged periods of aerobic floating that the production of inhibitor is reduced sufficiently to prevent effective concentrations from again arising in large spore populations.

Since the depletion of inhibitor can occur without concomitant depletion of the reserves required for germination, 2 different spore reserves may well be concerned, 1 of which is used up long before the other. Such an interpretation is consistent with the observation that spores depleted of inhibitor are still capable not only of germinating, but even of forming germ tubes comparable in size with those formed when untreated spores escape the action of inhibitor and germinate soon after contact with liquid. It is also possible, however, that the formation of inhibitor and growth of germ tube both depend upon the same ultimate spore reserve, but that this reserve is transformed during the pre-floating period into an intermediate which is still a good source of material and energy for germ tube formation but is not a satisfactory inhibitor precursor. The very rapid germination of pre-floated spores suggests that some mobilization of reserves has, in fact, taken place while germ tube formation was under suppression by the inhibitor. In any event, the point of action of the inhibitor must be a reaction that is of equal importance to germination whether the spores have been pre-floated or not.

Information concerning the mode of action of the inhibitor can best be obtained when purified preparations of the material are available. However, the fact that DNP is an effective counteractant in concentrations that prevent synthetic reactions in other organisms and are effective in uncoupling phosphorylation from oxidation, suggests that the counteraction could involve the release of phosphate from organic complexes, and hence that the initial effect of the inhibitor might be to cause an accumulation of organic phosphate complexes that are not available for germination.

The participation of an inhibitor arising from the uredospores themselves adequately accounts for the erratic germination of untreated uredospores. The final emergence of a germ-tube is subject not only to the direct effects of environment upon the mobilization of spore reserves, but also to the indirect effects exerted through altered production, retention, or effectiveness of the inhibitor. Thus small changes, which would not appreciably affect any of the processes involved in germination in the absence of inhibitor, can play a major role when the effectiveness of the inhibitor is also concerned. For example, inhibitor-depleted spores germinate readily whether submerged or floating, provided they are not in contact with a solution of inhibitor, whereas untreated spores do not germinate when submerged. Since submergence would lead to a similar reduction in the access of O_2 for both types of spores, these results suggest strongly that it is not a lack of O_2, as previously suggested (3, 7), but the presence of inhibitor that prevents submerged spores from germinating.

The effectiveness of a propagative structure depends not only on its capacity for renewed development, but also on its capacity for refraining from development except under circumstances which favor successful propagation of the species. The restraint upon germination is exercised by inhibitors originating from the parent plant in some seeds (11), pollen grains (19, 20), and fungus spores (2), and probably by substances from the spores themselves in some other instances (4, 8, 9, 25). Other substances, capable of counteracting these inhibitors, would relieve the restraint upon development, and by their distribution could therefore determine the loci where development would be permitted.

If the readiness with which uredospores germinate plays a crucial role in establishing infection, the interplay between self-inhibitor and counteracting substances (or adsorbing surfaces) in the host may be a determining factor in the occurrence of stem rust.

Department of Botany
University of Wisconsin
Madison, Wisconsin

LITERATURE CITED

1. Anderson, J. A. 1934. Studies on the nature of rust resistance in wheat. VI. Effect of hydrogen ion concentration, phenolic compounds, and host extracts on the germination of urediniospores of Puccinia graminis tritici, form 21. Can. Jour. Res. **11**: 667-686.
2. Backus, M. P. 1939. The mechanics of conidial fertilization in Neurospora sitophila. Bul. Torr. Bot. Club **66**: 63-76.
3. Beauverie, J. 1924. Sur la germination des urédospores des rouilles du blé. Compt. Rend. Acad. Sci. Paris. **179**: 993-996.
4. Boyd, A. E. W. 1952. Dry rot disease of the potato. IV. Laboratory methods used in assessing variations in tuber susceptibility. Ann. Appl. Biol. **39**: 322-329.
5. Brown, R. 1946. Biological stimulation in germination. Nature **157**: 64-69.
6. Carleton, M. A. 1893. Studies in the biology of the Uredineae. Notes on germination. Bot. Gaz. **18**: 447-457.
7. De Bary, A. 1865. Neue Untersuchungen uber die Uredineen insbesondere die Entwickelung der Puccinia graminis und den Zusammenhang derselben mit Aecidium Berberidis. Monatsber. K. Akad. d. Wiss.
8. Doran, W. L. 1922. Effect of external and internal factors on the germination of fungous spores. Bul. Torr. Bot. Club **49**: 313-340.
9. Duggar, B. M. 1901. Physiological studies with reference to the germination of certain fungous spores. Bot. Gaz. **31**: 38-66.
10. Eriksson, J. u. E. Henning. 1896. Die Getreideroste. Stockholm.
11. Evenari, M. 1949. Germination inhibitors. Bot. Rev. **15**: 153-194.
12. Ezekiel, W. N. 1930. Studies on the nature of physiologic resistance to Puccinia graminis tritici. Univ. Minn. Agr. Exp. Sta. Tech. Bul. **67**: 1-62.
13. Hitchcock, A. S., and M. A. Carleton. 1893. Preliminary report on rusts of grain. Bul. Kans. Agr. Exp. Sta. **38**: 1-14.
14. Hursh, C. R. 1922. The relation of temperature and hydrogen ion concentration to urediniospore germination of biologic forms of stem rust of wheat. Phytopathology. **12**: 353-361.
15. Mains, E. B. 1915. Some factors concerned in the germination of rust spores. Rep. Mich. Acad. Sci. **17**: 136-140.
16. Melander, L. W. 1931. The effect of temperature and light on the development of the uredinial stage of Puccinia graminis. (Abs.) Phytopathology. **21**: 109.
17. Melhus, J. E., and L. W. Durrell. 1919. Studies on the crown rust of oats. Iowa Agr. Exp. Sta. Res. Bul. **49**: 115-144.
18. Schaffnit, E. 1909. Biologische Beobachtungen uber die Keimfähigkeit und Keimung der Uredo- und Aecidiosporen der Getreideroste. Ann. Mycol. **7**: 509-523.
19. Schwarzenbach, F. H. 1951. Die Wirkung von Carotinoider auf die Keimung von Cyklamenpollen. Helv. Chim. Acta **34**: 1064-1069.
20. Schwarzenbach, F. H. 1953. Carotinoide als Wirkstoffe der Fortpflanzungs-physiologie von Cyclamen persicum Mill. Vierteljahrsschrift der Naturforsch. Gesellschaft in Zurich **98**(1): 1-49.
21. Sibilia, C. 1928. Richerche sulle ruggini dei cereali. Boll. R. Staz. Pat. Veg., n.s. **85**: 235-247.
22. Stock, F. 1931. Untersuchungen uber Keimung und Keimschlauchwachstum der Uredosporen einiger Getreideroste. Phytopath. Zeits. **3**: 231-279.
23. Tervet, I. W., and R. C. Cassell. 1951. The use of cyclone separators in race identification of cereal rusts. Phytopathology **41**: 286-290.
24. Ward, H. M. 1902. On the relations between host and parasite in the bromes and their brown rust, Puccinia dispersa (Erikss.) Ann. Bot. **16**: 233-315.
25. Domsch, K. H. 1954. Keimungsphysiologische Untersuchungen mit Sporen von Erysiphe graminis. Arch. Mikrob. **20**: 163-175.

PATHOLOGICAL ANATOMY OF BACTERIAL CANKER OF YOUNG TOMATO PLANTS [1]

T. S. Pine, R. G. Grogan, and Wm. B. Hewitt

SUMMARY

A detailed study of serial sections of young tomato plants infected with *Corynebacterium michiganense* revealed that initial infection and subsequent movement of the organism occurred in the spiral vessel elements of the primary xylem when inoculated through cut roots or petioles. However, 5 or more days after inoculation, bacteria were found in pockets about the vessels and in some instances the pockets had extended inward to involve the internal phloem and pith. The expansion of bacterial pockets in the phloem tissue was relatively slow and occurred in the same manner as in the xylem and pith parenchyma. No evidence of extended longitudinal movement of the organism in the phloem tissues was observed.

In the original reports concerning bacterial canker of tomato by Smith (14, 15), *Corynebacterium michiganense* (E.F. Sm.) Jensen was described and illustrated as being primarily a phloem parasite. Miss Bryan (1) accepted this interpretation in 1928. However, in 1930 (2) she stated that the bacteria appeared first in the inner vessels of the wood and spread from there to the more succulent phloem, pith, and cortex. No detailed illustrations were presented nor was any explanation given for the apparent contradiction between the 2 accounts. Since 1930 Dufrenoy and Shapovalov (5), Kendrick and Walker (10), Walker and Kendrick (16), and Mushin (11), have accepted Smith's interpretation, while Fish and Pugsley (6) and Cass Smith and Goss (3) have expressed the opinion that this canker organism is primarily a xylem parasite. Dowson (4) reported that bacteria were confined at first to both the xylem and phloem of the conducting tissue but are easily demonstrated in wilting shoots where they are chiefly present in the spiral vessels of the earliest-formed wood. It is difficult to determine with certainty whether these later workers were interpreting their own observations, or were influenced by Miss Bryan's 1930 account.

No detailed illustrations or accounts of the pathological anatomy of bacterial canker of tomato have appeared in the literature since the original work of Smith. The present paper is a report resulting from a detailed study of the pathological anatomy of bacterial canker of tomato with special emphasis on the tissues involved in the early stages of disease development and in the movement of the bacteria to determine whether or not phloem tissues are involved in longitudinal spread of *C. michiganense*.

MATERIALS AND METHODS.—Five-week old seedling tomatoes of the variety Improved Pearson growing in 6-in. pots were used throughout this study. One lot of plants was not inoculated. A second lot was inoculated by severing a leaf petiole at the point of attachment to the main axis with a scalpel dipped in a water suspension of *Corynebacterium michiganense*. The bacterial suspensions were made by pouring sterile distilled water over 4-day-old potato dextrose agar cultures. A third lot of plants was inoculated by pouring a bacterial suspension onto the soil around young healthy seedlings, and then cutting the roots at 4 points equidistant around the crown at about 1 in. from the crown.

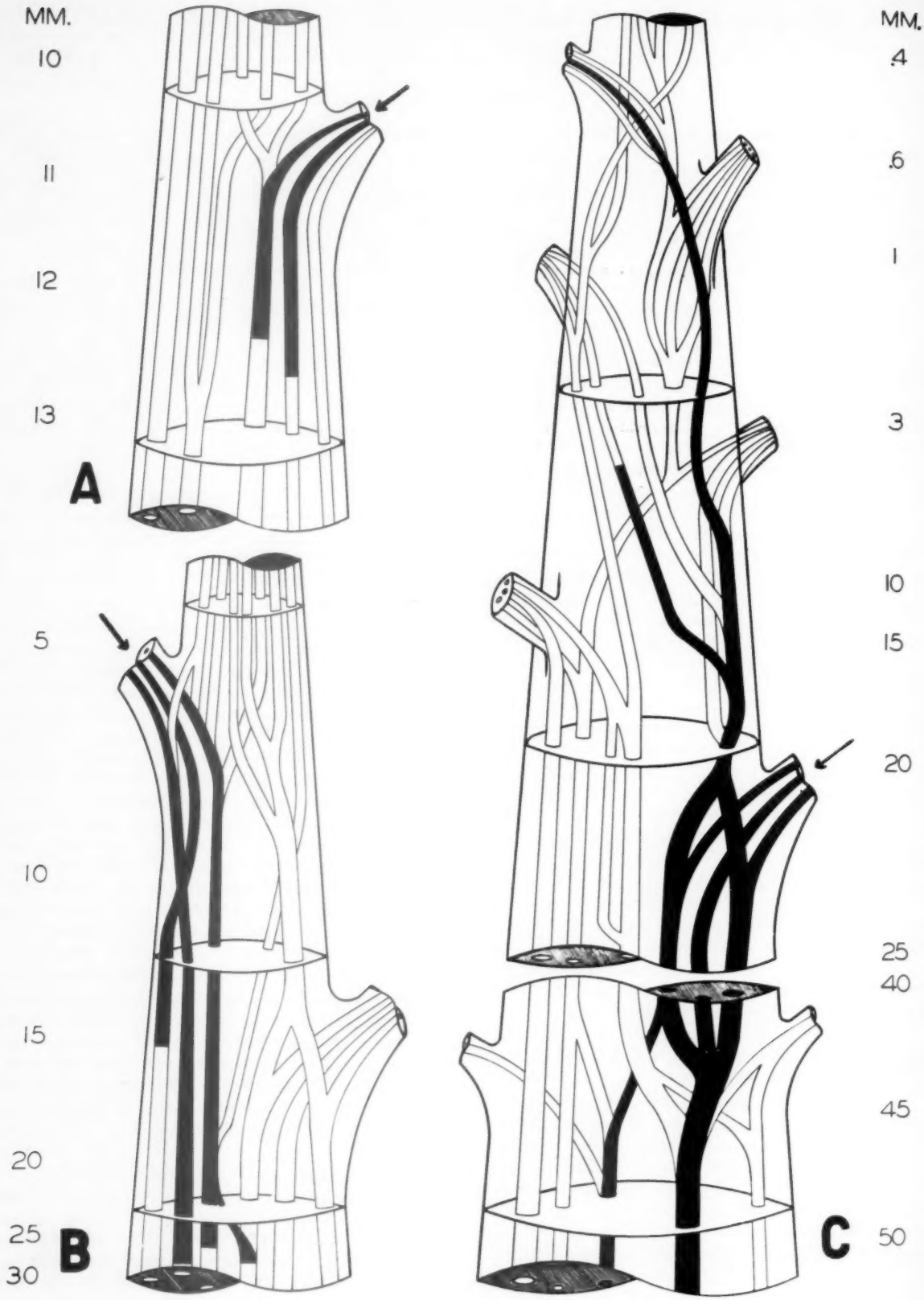
Five plants were collected from each lot 15 minutes and 1, 3, 5, 7, 9, and 18 days, respectively, after inoculation. All plants were killed and fixed in Karpachenco's solution (13) then infiltrated and imbedded in paraffin by means of the n-butyl alcohol technique described by Johansen (9).

For detailed study, 1 plant was chosen at random from the petiole-inoculated series collected 15 minutes and 1, 5, and 9 days after inoculation. This material was sectioned serially at approximately 10 μ from the apex to a point about 50 mm down the main axis. The slides were stained with safranin 0 and fast green. After examination of this material, a portion of each of the remaining plants in these lots and in the soil-inoculated group was sectioned, stained, and studied to determine whether or not any difference existed between these plants and the ones which were studied in more detail. Since no essential differences were observed, the descriptions of the pathological anatomy presented in the text which follows were made from the plants which were examined in greatest detail.

EXPERIMENTAL RESULTS.—When the leaf petiole of a young tomato plant was cut off at a point close to the main axis with a scalpel wet with a water suspension of *C. michiganense*, the bacteria moved through the vascular elements of the leaf traces into the main axis of the plant. Different stages of this movement are shown schematically in Figure 1 for plants collected 15 minutes and 1 and 5 days after inoculation.

Fifteen minutes after inoculation, bacteria were found in the spiral vessel elements of only 2 leaf traces, at a distance of 2.33 mm below the cut surface of the leaf petiole (Fig. 1, A). All visible bacteria were confined to the vessel elements. They appeared in clumps and were lodged between the spiral thickenings of the vessel walls.

[1] Accepted for publication December 20, 1954.


MM.
10
11
12
13
A
5
10
15
20
25
30
B
MM.
4
6
1
3
10
15
20
25
40
45
50
C

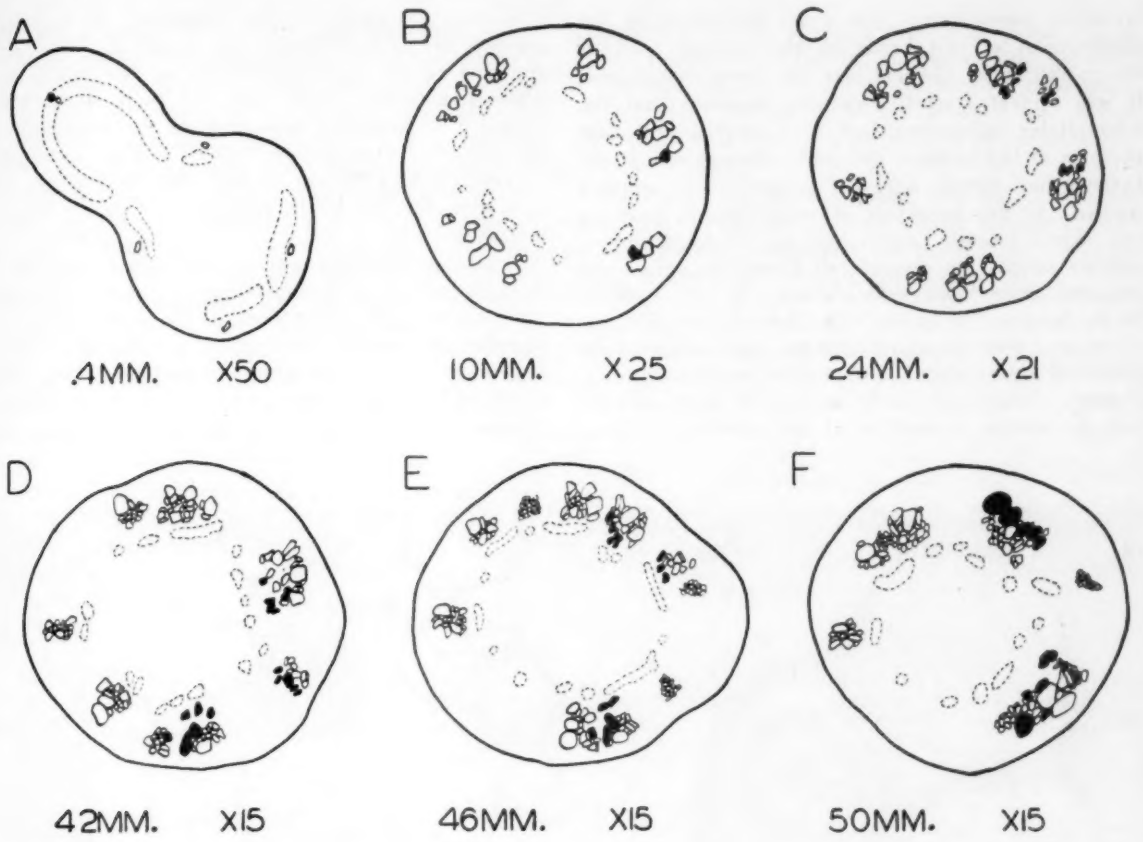


FIG. 2. Cross sections of the main axis of a young tomato plant collected 5 days after inoculation with the bacterial canker organism. Solid black areas indicate the positions of bacteria in and around xylem elements of the primary vascular system. These sections were taken from the plant diagrammed in Fig. 1, C at measured distances below the apex, corresponding to the measurements given in Fig. 1, C.

In the plants collected 1 day after inoculation, bacteria were found in all 3 traces of the inoculated petiole and at points down the main axis for a distance of 24.5 mm below the cut petiole surface (Fig. 1, B). Although these traces which contained bacteria made direct contact with 6 other vascular bundles, it was only at the lowermost connection that bacteria had spread into a connecting vessel. Bacteria were found only in the vessel elements of the xylem and none were found above the point of inoculation. Despite the large increase in the mass of bacteria, which nearly filled the lumina of some vessels, there was no evidence of any movement outward into other tissues from these vessels.

In the plant collected 5 days after inoculation, bacteria had moved freely through the spiral vessels and had spread laterally, forming pockets in the surrounding tissues (Fig. 3, D). Bacteria were observed at points 13.2 mm above the point of inoculation and at more than 32.5 mm below this point (Fig. 1, C).

The most extensive movement of the organism took place in the spiral vessel elements. In areas near the point of inoculation, bacteria were confined to the vessels (Fig. 3, A). However, in plants collected 5 or more days after inoculation, bacteria were found in pockets about the vessels. Some such pockets had extended inward to the internal phloem and pith (Fig. 3, D). Bacterial pockets, studied in serial sections, showed a progressive development. The bacteria moved through cavities in the thin primary cell walls of vessels into the intercellular spaces of

FIG. 1. Extent and path of movement of bacteria in young tomato plants inoculated with *Corynebacterium michiganense* by severing a leaf petiole at the point of attachment to the main axis. Plants collected: A) 15 minutes, B) 1 day, and C) 5 days after inoculation. Arrows indicate the points of inoculation. The solid black areas indicate primary vascular bundles in which bacteria were observed. Cross sections of plant C are shown in Fig. 2.

bordering parenchyma. The exact mechanism of the development of this break in the primary wall of the spiral vessel element has not been determined. It was evident from the staining reactions that the intercellular substances had been altered and that portions of the primary cell wall, through which the bacteria had moved, were not present in the sections studied. In the formation of some pockets, bacteria had moved laterally and longitudinally in the intercellular spaces for several cell diameters before the adjacent parenchyma cells showed any visible effect. In the progress of pocket formation, the intercellular substances were dissolved, and the parenchyma cells appeared to be isolated in the cavity before collapsing. Portions of cell walls could be found in some pockets, but the precise disposition of the cellulose of these cells was not evident. The expansion of bacterial pockets in phloem tissue was relatively slow and occurred in the same manner as in the xylem and pith parenchyma. At no time was there evidence of extended longitudinal movement of the organism in the phloem tissue or sieve tubes. Lateral movement of bacteria into the cortex was observed, developing at a slow rate and in the same manner as the inward movement.

Examination of root sections from plants inoculated through the soil showed that bacteria moved from the cut roots into the plant through the xylem elements, and that subsequent development of infection in other root tissues occurred in a manner similar to that which occurred when inoculation was through a cut petiole.

When the leaf petiole of a young tomato plant was

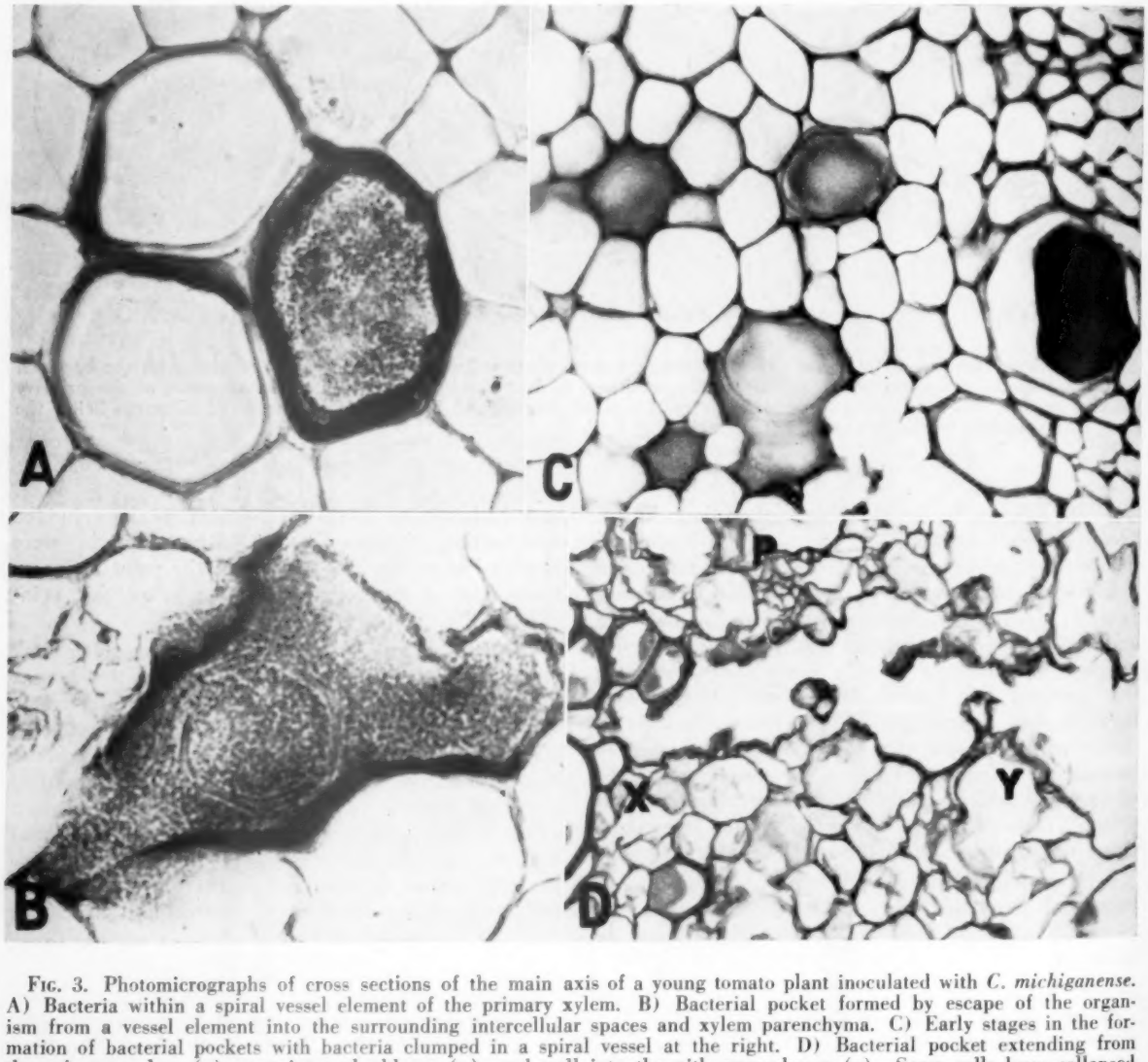


FIG. 3. Photomicrographs of cross sections of the main axis of a young tomato plant inoculated with *C. michiganense*. A) Bacteria within a spiral vessel element of the primary xylem. B) Bacterial pocket formed by escape of the organism from a vessel element into the surrounding intercellular spaces and xylem parenchyma. C) Early stages in the formation of bacterial pockets with bacteria clumped in a spiral vessel at the right. D) Bacterial pocket extending from the primary xylem (x), past internal phloem (p), and well into the pith parenchyma (y). Some cells have collapsed completely, while others float about within the cavity.

cut with a scalpel dipped in India ink instead of a bacterial suspension, it was possible to follow the path and probable extent of the initial movement of bacteria at the time of inoculation (8). Shortly after the leaf petiole was cut with a scalpel dipped in India ink, plants were dissected and the extent of movement of the ink particles was determined. Some plants were cleared in lacto-phenol before examination. Examination revealed that ink particles had moved into the spiral vessels of all traces, from about 10 mm to over 45 mm and averaged about 30 mm. This follows fairly closely the extent of bacterial development found in the plants collected 1 day after inoculation. This suggests that bacteria may have been drawn into the vessels to approximately the same extent at the time of inoculation, but that the staining technique used did not make it possible to detect them beyond 2.33 mm.

After the initial intake, the bacteria increase and partially fill the elements and extend to a greater distance down the cut vessels before there is any spread to lateral bundles, or any upward movement. Small clumps of cells lodge between the spiral thickenings where they multiply rapidly and eventually fill the lumina. At these points of accumulation, the primary cell walls are affected and lateral spread is initiated. Where 2 vessel elements lie adjacent in the same bundle, the spread is most rapid. When the vessel element of an inoculated bundle in which the flow of bacteria is downward lies adjacent to a similar element of a noninfected bundle, spread to the latter vessel results in a rapid upward movement of bacteria. This transfer of the pathogen between adjacent xylem bundles and the retention of the organism within these bundles tends to explain the one-sided wilting symptom characteristic of bacterial canker of tomato, and is the reason for the relatively rapid development of this symptom.

Discussion.—It can be argued that the technique of inoculating by cutting the leaf petiole is purely artificial and that the bacteria are in this way placed directly in the xylem elements where one might expect rather rapid and extensive movement. On the other hand, if the bacteria developed and moved better in any of the other leaf petiole tissues, such as the phloem, these tissues were equally opened to entry and subsequent development by this method of inoculation. The same objection might be made against inoculation by cutting roots, or injuring other parts of the tomato plant. However, it has been shown by Grogan and Kendrick (7) and Orth (12) that the bacterial canker organism is strictly a wound parasite and that infection occurs only through wounds. Although infections take place in cortical wounds, a much higher percentage of infection and much more rapid disease development occurs as a result of wounds that extend into the xylem.

The long, open tubular structure of the spiral elements of the primary xylem system in the tomato plant permits the rapid and extensive movement of the bacteria. Bacteria lodge between the spiral thickenings, where they increase and often fill or bridge the lumen of the vessel. Apparently products of bacterial action at such places cause a dissolution of the pectic substances of the primary wall which allows the bacteria to ooze into the adjacent intercellular spaces.

The sieve tube of the phloem does not permit the movement of bacteria. These studies emphasize this point and clearly show that the bacteria do not move about freely in the phloem tissue.

Department of Plant Pathology
University of California
Davis, California

LITERATURE CITED

1. Bryan, Mary K. 1928. Bacterial canker of tomatoes. U. S. Dept. Agr. Cir. 29, 4 p.
2. Bryan, Mary K. 1930. Studies on bacterial canker of tomato. Jour. Agr. Res. **41**: 825-851.
3. Cass Smith, W. P., and O. M. Goss. 1946. Bacterial canker of tomatoes. Jour. Dept. Agr. West. Austral. **23**: 147-156.
4. Dowson, W. J. 1949. Gram-positive bacterial plant pathogens: Corynebacterium. In his Manual of bacterial plant pathogens. Adam and Charles Black, London, p. 68-71.
5. Dufrenoy, J., and M. Shapovalov. 1936. The histological and cytological reactions of tomatoes to infection by Aplanobacter michiganense E. F. Smith. [Paris] Soc. de Biol. Compt. Rend. **123**: 695-696.
6. Fish, S., and A. T. Pugsley. 1936. Bacterial canker of tomatoes. Victoria Dept. Agr. Jour. **34**: 520.
7. Grogan, R. G., and J. B. Kendrick, Sr. 1953. Seed transmission, mode of overwintering and spread of bacterial canker of tomato caused by Corynebacterium michiganense. (Abs.) Phytopathology **43**: 473.
8. Hewitt, Wm. R. 1938. Leaf-scar infection in relation to the olive-knot disease. Hilgardia **12**: 41-71.
9. Johansen, D. A. 1940. Paraffin methods. In his Plant microtechnique. Ed. 3. McGraw-Hill, New York. p. 130-132.
10. Kendrick, J. B. Jr., and J. C. Walker. 1948. Predisposition of tomatoes to bacterial canker. Jour. Agr. Res. **77**: 169-186.
11. Mushin, Rose. 1938. Studies in the physiology of plant pathogenic bacteria. The food requirements of a xylem invader, Bacterium solanacearum E. F. S., and a phloem invader Aplanobacter michiganense E. F. S. Austral. Jour. Exp. Biol. and Med. Sci. **16**: 323-329.
12. Orth, H. 1937. Untersuchungen uber die Biologie und Bekämpfung des Erregers der Bakterienwelke der Tomaten (Bact. michiganense E. F. S.). Zentbl. f. Bakt. Abt. II, **96**: 376-402.
13. Rawlins, T. E. 1933. Microscopic methods. In his Phytopathological and botanical research methods. Wiley, New York. p. 13.
14. Smith, E. F. 1914. The Grand Rapids tomato disease. In his Bacteria in relation to plant diseases. Carnegie Institution, Washington. v. 3, p. 161-165.
15. Smith, E. F. 1920. Bacterial canker of tomato. In his Introduction to bacterial diseases of plants. Saunders, Philadelphia and London. p. 202-222.
16. Walker, J. C., and J. B. Kendrick, Jr. 1948. Plant nutrition in relation to disease development, IV. Bacterial canker of tomato. Amer. Jour. Bot. **35**: 186-192.

A MOSAIC DISEASE OF HIMALAYA BLACKBERRY[1]

Stanley M. Alcorn, Stephen Wilhelm, H. Earl Thomas[2]

SUMMARY

A mosaic disease, possibly caused by a virus complex, is ultimately lethal to Himalaya blackberry. The causal agent is transmissible by grafting to red raspberry and to peach. It appears not to be transmitted through Himalaya seed. Symptoms in peach indicate a relation to yellow bud mosaic of peach.

In 1945, attention was called to a mosaic disease in a single planting of Himalaya blackberry (*Rubus procerus* P. J. Muell.), about 27 years old, in central coastal California. At that time, 35 plants were affected visibly or missing in 1 compact area in an otherwise uniform stand. By 1952, the area involved at least 128 affected or missing plants and a few foci consisting of 1–5 plants had appeared outside the general area. Spread has been more rapid down a slope than upward and still more rapid laterally with the rows and in the direction of cultivation, indicating a vector of limited independent movement.

SYMPTOMS.—Under both field and greenhouse conditions, diseased plants exhibit a variety of leaf symptoms in Himalaya blackberry. These include small irregularly shaped, chlorotic spots scattered over the leaf blade, large yellow blotches at the base of the leaflet, conspicuous veinal chlorosis, and oak–leaf patterns (Fig. 1, A, C). Chlorotic areas may be delimited sharply from the surrounding tissue, or the chlorosis may grade gradually into the natural green color of the leaflet. Feather vein is used here to denote the type of veinal chlorosis extending from large to small veins, producing a feathery appearance (Fig. 1, B). This symptom is associated with the disease under consideration but may occur also independently in Himalaya and other blackberry varieties. The most common symptom combination in the Himalaya variety seems to be that of basal chlorosis of the leaflets and small chlorotic blotches (Fig. 1, C). Distortion and rugosity of the lamina often are associated symptoms. In many affected leaves, chlorosis may predominate, leaving only dispersed islets of green (Fig. 1, A). As the disease progresses, affected plants show a marked reduction in leaf size, number of leaflets (Fig. 1, A), cane length, and cane diameter. The end result in the field is death of the plant.

Affected plants transplanted from the field to pots usually died without resuming growth. Occasionally, however, such plants survived for several years.

SUSCEPT RANGE.—Plants of the Boysen, Nectar, and Young varieties; seedlings of Chehalem and Logan blackberries; and the native California blackberry (*R. ursinus* Cham. & Schlect.) when inarch-grafted to affected Himalaya blackberry have shown to date only the symptoms of the feather–vein (Fig. 1, B) or oak–leaf type rather than the entire range of symptoms displayed by the Himalaya variety. Only feather–vein symptoms have been noted in plants of Himalaya or other commercial blackberry varieties inoculated from such feather–vein plants.

Leaves of the inoculated Lloyd George raspberry (*R. idaeus* L.) have shown marked contortion with chlorotic rings and blotches. Such symptoms were confined to the inoculated shoot and its lateral branches. The new growth formed the season following symptom expression was symptomless. The typical mosaic pattern was produced in a cane of Himalaya variety when it was inarched to a shoot of Lloyd George variety showing symptoms but not when the inarch was made later to a symptomless cane on the same plant.

Symptoms suggestive of virus developed in all of 8 Lovell peach seedlings inarch-grafted to affected plants of the Himalaya blackberry. Twenty–two of 33 peach seedlings subinoculated from these Lovell seedlings also showed similar symptoms. In all instances, symptoms were first observed the season following that of inoculation. On peach, lateral buds near the point of inoculation often failed or died after starting to grow, or produced compact clusters of dwarfed, misshapen leaves (Fig. 1, D; stem at right). Growth from buds a few inches distant from the point of inoculation usually appeared normal. Some of the first-formed leaves, however, were misshapen, with chlorotic areas appearing typically along the midvein in the region of distortion and occasionally along lateral veins (Fig. 1, D). Such chlorotic areas usually died and dropped out. Later-formed leaves appeared normal. In subsequent years, more of the tree became severely affected until the entire tree was involved. Occasionally small seedlings were affected throughout during the first season of symptom appearance.

Equally successful inoculation from peach to peach may be effected with inoculum taken either from the stem or from larger roots of an affected plant. Mosaic

[1] Accepted for publication December 15, 1954.

[2] Respectively, former Research Assistant and at present Merck and Company. Postdoctoral Fellow in Plant Pathology in the Department of Plant Pathology; Assistant Professor; and Professor of Plant Pathology, University of California, Berkeley 4, California.

symptoms have been noted in 1 of 4 Himalaya plants inarched to affected peach seedlings. In 1 of 2 attempts to transmit the virus from plants of Himalaya to almond, a few of the first-formed leaves on a single

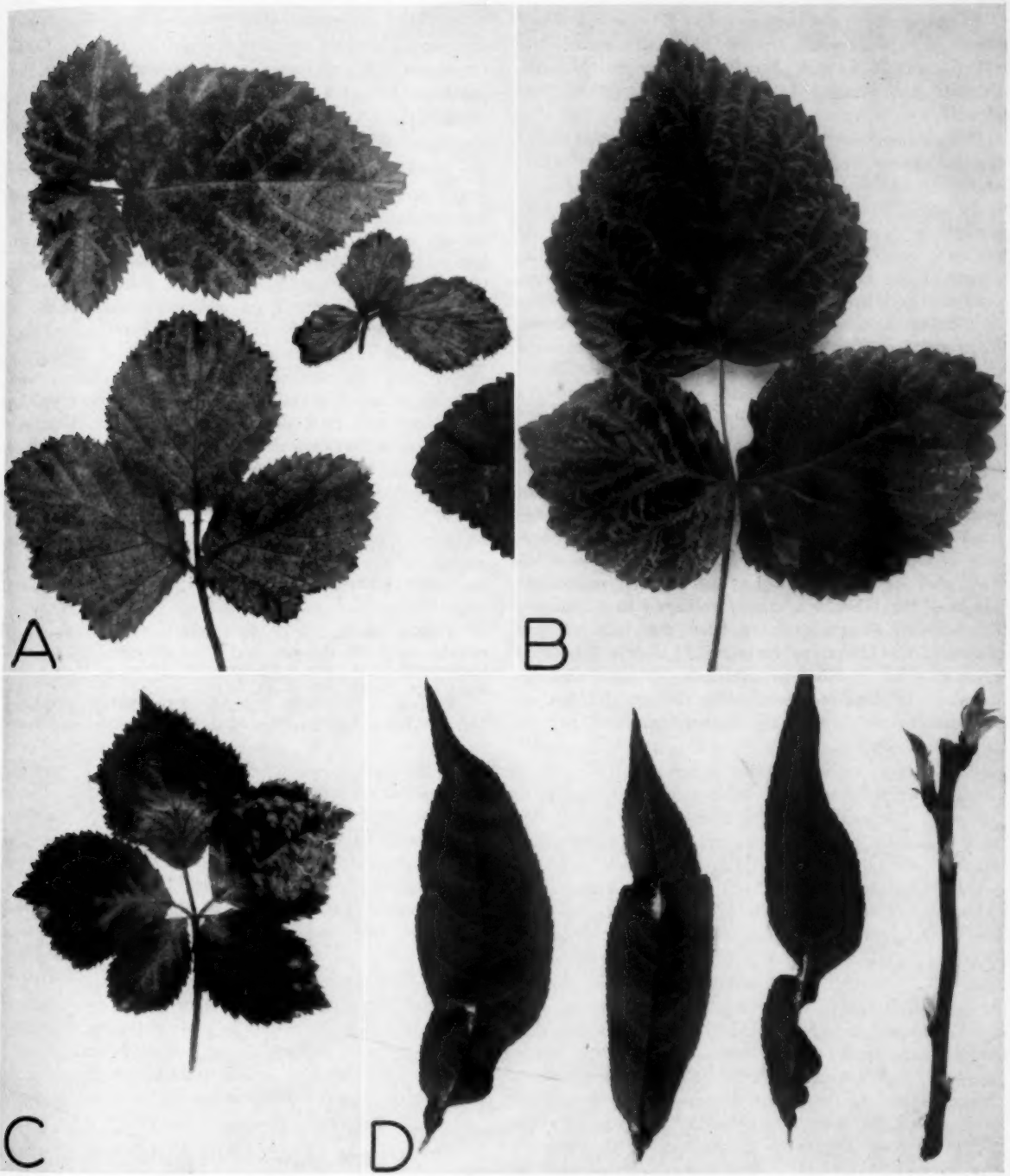


FIG. 1. A) Himalaya blackberry leaves severely affected with Himalaya mosaic. Leaflet number has been reduced and leaf size diminished (leaf, center right). B) Feather-vein leaf symptoms in loganberry inarch-grafted to infected Himalaya. C) Mosaic symptoms on a moderately affected Himalaya blackberry leaf. Note the feathery-edged basal leaflet chlorosis associated with laminar blotches. D) Symptoms in peach produced by inoculation from mosaic Himalaya. At right, retarded chlorotic leaves from buds near site of inoculation; at left, distortion and chlorotic blotches along larger veins of leaves more distant from inoculation site.

branch of the Peerless almond showed slight distortion and chlorosis along the main vein in the region of distortion, in the second growing season after inoculation.

Symptoms have not been noted to date on inoculated plants of apple, apricot, cherry (Mahaleb), pear, plum (Marianna 2624 and Myrobalan), rose (Manetti, Odorata, and Ragged Robin), and *Rubus leucodermis* Dougl.

Other observations.—The minimum period noted for the appearance of the mosaic symptoms in plants of the Himalaya variety under greenhouse conditions is 39 days. Rooted tips of canes from affected plants sometimes develop symptoms even when the tips do not have symptoms at the time of rooting. Six healthy plants of the Himalaya variety transplanted to large pots with soil from the affected area remained healthy up to 26 months, when the last observations were made. However, 1 of 8 peach seedlings developed symptoms characteristic of the disease when these were planted in cans of garden soil each with an affected Himalaya plant recently brought in from the field.

Of 146 seedlings of the Himalaya variety grown from seed from affected plants none developed typical symptoms. A few showed mild feather-vein or oak-leaf markings, but about an equal proportion of seedlings from seemingly healthy plants did likewise. Feather-vein symptoms have been produced in Boysen or Youngberry plants inarched to apparently symptomless plants of the Himalaya variety collected in 3 counties. Feather-vein symptoms have been noted in escaped plants of the Himalaya variety in 2 widely separated counties. In 9 different attempts, peach seedlings inarched to feather-vein-affected Boysen, Nectar, or Youngberry plants have not shown symptoms.

Because of the similarity of symptoms of the Himalaya disease in peach to the yellow bud mosaic of peach[3], preliminary attempts were made to infect plants of the Himalaya variety with the yellow bud virus. Mild symptoms were produced including veinal chlorosis, chlorotic rings and slight distortion. These symptoms did not recur in the second year but the yellow bud virus was recovered in peach in both years.

Discussion.—With the possible exception of a condition noted in Himalaya blackberry by Blodgett[4], the mosaic described here does not appear to have been encountered elsewhere. Limited observations of the disease in raspberry are not of sufficient scope to permit comparison with the known diseases of raspberry. In peach, the symptoms show some resemblance to those of yellow bud mosaic[3]. The disease caused in peach by inoculation from mosaic-affected plants of Himalaya variety, however, has a much longer incubation period than that of the yellow bud mosaic, a slower rate of movement through the plant, and possibly a more drastic local effect adjacent to the inoculation site. Suscept range studies of the Himalaya disease and the variation in symptoms in plants of the Himalaya variety indicate at least 2 component viruses.

Department of Plant Pathology
University of California
Berkeley 4, California

[3] Thomas, H. E., and T. E. Rawlins. 1951. Yellow bud mosaic. In Virus diseases and other disorders with virus-like symptoms of stone fruits in North America. U. S. Dept. Agr., Agr. Handb. 10: 53-55.

[4] Blodgett, E. C. 1942. Diseases of small fruits in Idaho. Univ. of Idaho Agr. Exp. Sta. Bul. 246, 27 p.

ADULT PLANT RESISTANCE TO CROWN RUST OF CERTAIN OAT SELECTIONS [1]

M. D. Simons[2]

SUMMARY

A number of lines of oats that showed adult plant resistance to crown rust were selected from a preliminary field test in 1953. In greenhouse tests the following winter, 27 of these lines were susceptible to crown rust race 202 in the seedling stage. These susceptible seedlings were grown to the heading stage and again tested for reaction to race 202. The upper and lower leaves of some plants were resistant, only the lower leaves were resistant on others, while still others showed no resistance.

In 1954, a succession of plantings of the 27 lines in the field were timed so that their reactions to crown rust in the first-leaf, tillering, heading, and soft-dough stages of growth could be determined simultaneously. All were susceptible in the first-leaf stage. In general, those that showed an appreciable degree of resistance in the 2 later stages of growth also were resistant in the tillering stage.

Several lines that were classified as resistant or highly resistant in the field in 1953 were susceptible in 1954.

The correlation between adult reaction in the field and reaction of plants in the heading stage in the greenhouse was complicated by the variation in susceptibility of different leaves of the greenhouse plants. In all cases, however, where the upper leaves were resistant in the greenhouse, the selection was resistant in the field.

The 3 selections showing the highest degree of adult resistance were P.I. numbers 174544, 174545, and 185783. P.I. numbers 189628, 190584, 193040, 197278, and 197279 showed a high degree of resistance.

There are many degrees and patterns of resistance to crown rust (*Puccinia coronata* Cda. var. *avenae* Fraser & Led.) in oats. However, the majority of the varieties and selections of oats now known to be resistant to crown rust may be roughly classified as having either "seedling resistance" or "adult plant resistance." In this study, those classified as resistant as seedlings retained their resistance in later stages of growth. Those with only adult plant resistance were susceptible as seedlings, but showed some degree of resistance as they grew older. At present, plant breeders and pathologists are forced to rely upon a small number of genes for seedling resistance in breeding new varieties resistant to crown rust. Experience has shown that these genes for resistance may be rendered useless in a few years by the appearance and rapid increase of races of crown rust capable of overcoming the resistance conferred by these genes in both seedling and adult plants. This investigation was an effort to find additional genes for adult plant resistance that might be useful in supplementing or replacing the seedling resistance now used in breeding rust-resistant oats.

Peturson (6), who reviewed the earlier literature dealing with adult plant resistance of oats, tested the reactions of 5 varieties of oats to 7 races of crown rust in the seedling and heading stages. All 5 varieties were susceptibile to all the races in the seedling stage. The Erban and Ajax varieties were resistant as adult plants to some of the races. The R.L. No. 1370 and R.L. No. 1510 varieties were resistant to all the races in the adult stage. Resistance in these 4 varieties was not uniform throughout the plant, the upper leaves being more susceptible than the lower. Simons (8) showed that the variety Mo. 0-205 was susceptible to race 205 in the seedling stage, but developed resistance to this race in later stages of growth. The stage at which resistance appeared varied with the temperature. The Appler, Marion, and Cherokee varieties, also susceptible in the seedling stage, showed moderate adult plant resistance at 15°C, but not at 25°C.

Several investigators (1, 4, 7) recently reported a number of lines and selections of oats to be resistant to crown rust under field conditions. Some of these varieties have been used in this study.

METHODS AND MATERIALS.—Methods of inoculation and notation of reaction type were similar to those described by Murphy (5) and Finkner, Atkins, and Murphy (2). Seed was obtained through David J. Ward, Germplasm Maintenance and Evaluation, Field Crops Research Branch, A.R.S., U. S. Dept. of Agriculture, Beltsville, Maryland.

SELECTION OF MATERIAL.—The World Collection of oats was grown in the field at Ames, Iowa, in 1953, where it was subjected to an extremely heavy, naturally-initiated epiphytotic of crown rust. A large number of varieties and selections, many of course, carrying genes from known sources of resistance, were observed to be resistant. From this material about 40 lines (all designated by P.I. numbers) were selected for further study. These had either immune-, 0-, or 1-type reactions, or else had only a trace of 2-, 3-, or 4-type uredia.

Several of these lines, which were shown later to be susceptible as seedlings, were outstanding for adult

[1] Accepted for publication January 10, 1955.
Cooperative investigation between the Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and the Iowa Agricultural Experiment Station. Journal Paper No. J-2621 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1176.

[2] Plant Pathologist, Field Crops Research Branch, U.S.D.A.

resistance. P.I. 186475 (a selection of *Avena strigosa*) apparently was immune. Five others, P.I. numbers 174544, 174545, 174547, 185783, and 186472, exhibited only very low percentages of the highly resistant, 0-type flecking reaction.

GREENHOUSE TESTING.—Seed harvested from plants resistant in the field was used for greenhouse testing in order to avoid possible mixed reactions. These lines, along with 5 named varieties (Table 1), were tested in the first-leaf stage for reaction to crown rust race 202. Those that were resistant or moderately resistant at this stage of growth were discarded. The 27 lines that were fully or very nearly fully susceptible were supplied with artificial light, and grown until the latest lines were fully headed. There was only about a week spread in heading date between the earliest and the latest. The 2 or 3 plants in each of 4 pots of each selection were inoculated with crown rust race 202, after the plants were headed.

The average type of infection was recorded separately for the upper leaves (including the flag leaf) and for the lower leaves. The upper leaves were generally either clearly susceptible or clearly resistant, those of most of the selections being susceptible. The resistant strains were P.I. numbers 174544, 174545, 185783, and 190584. P.I. 174544 supported a few small uredia, the others were flecked, but had no uredia.

Infection on the lower leaves was somewhat irregular and a considerable range of reaction-types on the same leaf was common. On the whole, the lower

TABLE 1. *Average reaction*[a] *or range of reaction to crown rust of 27 selections and 5 varieties of oats in different stages of growth in the field and in the greenhouse*

Name or P.I. No.	Field 1953 adult	Greenhouse			Field 1954			
		Seedling	Heading, upper leaves	Heading, lower leaves	1st leaf	Tillering	Heading	Soft dough
Markton		4	4	4	4	100-4	100-4	90-4
Clinton		4	4	4	4	100-4	100-4	100-4
Landhafer		0	1,2,3	0	0,1	40-0,1	40-0,1	40-0,1
Mo. 0-205		4	3,4	0	4	80-4	60-3	60-3
Appler		4	4	3,4	4	30-0,2	30-0,2	30-3,4
P.I. 174544	Tr-0	4	0,1	0	4	10-0	5-0	5-0
P.I. 174545	Tr-0	4	0	0	4	5-0	5-0	5-0
P.I. 174547	5-0	4	4	2,3,4	4	60-4	30-4	60-4
P.I. 174563	30-0	4	4	0,2,3	4	60-2,3+	30-0,1	30-0,2
P.I. 177824	Tr-3	4	4	0	4	40-2,4	Tr-0,1	Tr-2,3
P.I. 177845	Tr-4	4	4	4	4	60-3,4	30-2,4	60-3,4
P.I. 183998	Tr-4	4	4	0	4	60-4	10-4	10-4
P.I. 185775	40-0	4	4	0	4	60-0,4	60-0,3+	30-0,3—
P.I. 185783	5-0	4—	0	0	3,4	10-0	10-0	20-0
P.I. 185790	Tr-4	4	4	0	4	100-4	40-0,3+	40-0,3
P.I. 185791	Tr-4	4	4	0	4	100-3,4	40-0,3+	40-0,3+
P.I. 185793	5-4	4	4	3,4	4	100-4	40-3,4	40-3,4
P.I. 186270	Tr-4	4	4	4	4	100-2,4	30-0,3	30-0,3
P.I. 186271	Tr-4	4	4	2,3,3+	4	40-0,3+	30-0,2	30-0,2
P.I. 186472	5-0	4	4	2,3	4	30-4	20-0,3	60-0,3+
P.I. 186473	50-1	4	3,4	0,1,2	4	30-2,3+	30-2,4	40-2,4
P.I. 186474	60-1	4	3,4	0	4	30-0,1	30-0,2	30-0,2
P.I. 186475	I	4—	3,4	0	4	60-0,2+	60-2,3	60-2,3
P.I. 189628	30-0	4	4	3,4	4	10-0,2—	20-0,2+	10-0,1
P.I. 189766	20-0	4	4	0	4	30-0,4—	30-0,4—	30-0,4—
P.I. 190584	Tr-4	4	0	0	4	40-2,3	40-0,1	30-0,1
P.I. 193040[b]	Tr-4	4	4	0	4	30-0,3	30-0,1	30-0,1
P.I. 197278	20-0	4	4	0	4	10-0,3	20-0	20-0
P.I. 197279	30-1	4	4	0,1,2	4	20-0	10-0	10-0
P.I. 197791	60-1	4	4	0,2,3	4	60-4	60-4	60-4
P.I. 197794	Tr-4	4	4	2,3,4	4	60-4	60-3+,4	60-3+,4
P.I. 198219	Tr-2	4	4	0	4	40-0,3	40-0,1	30-0,2

[a] For readings taken in the field, the figure preceding the hyphen represents the percentage of the leaf surface covered by uredia or flecks. The figures for greenhouse readings and the figures following the hyphen for field readings refer to type of infection as follows: I—no macroscopic evidence of infection; 0—necrosis or chlorosis, but no uredia; 1—necrotic or chlorotic areas, some of which contain small uredia; 2—necrotic or chlorotic areas, most of which contain small to midsized uredia; 3—chlorotic areas surrounding abundant, midsized uredia, no necrosis; 4—no necrosis or chlorosis, uredia large and abundant.

[b] Designated as Klein 69B, but apparently differs in crown rust reaction from the stock of Klein 69B used by Finkner (3).

leaves were more resistant than the upper leaves, and in no case were the lower leaves significantly less resistant than the upper. The lines with highly resistant lower leaves included those with upper leaves that were also highly resistant. Several other lines showed a striking difference between reaction-types of the upper and lower leaves, the complete susceptibility of the upper leaves grading rapidly into a high degree of resistance in the lower leaves. In still other lines the lower leaves showed various different infection types and the upper leaves were susceptible. Four selections showed no appreciable resistance in any leaves.

Field testing in 1954.—Seed harvested from plants tested for adult reaction in the greenhouse was used for field testing in 1954. In order to study the reaction of plants of different ages under similar conditions in the field, a 2-ft. row of each of the 27 lines was sown on each of 4 different dates: April 13 (which is about the normal seeding date), April 29, May 20, and June 11. Spreader rows of plants of a susceptible variety were inoculated with crown rust race 202. Variation from line to line in rate of growth and date of heading under field conditions was much greater than that in the greenhouse. Many lines showed some winter growth habit, but plants of most lines of the earliest seeding had headed when the first notes on crown rust reaction were made on July 1. The stage of growth of plants of the second sowing varied considerably at this time. Plants of the third sowing were mostly in the tillering stage, and those of the fourth sowing were uniform in size, when observations of the reactions of the first leaves were made.

All 27 lines were fully susceptible in the first-leaf stage. Thus the association of the seedling reactions of these lines between the greenhouse and the field was very close.

The different lines showed much variation in rust reaction as measured by their behavior in the tillering stage. Four, P.I. numbers 174544, 174545, 185783 and 197279, were highly resistant. Many showed a wide range in reaction types, while others were just as susceptible as in the first-leaf stage. There was a marked tendency in some lines for plants in this stage to be more heavily rusted, in terms of percentage of leaf area covered by uredia, than plants at the 2 later stages of growth.

There were few striking changes in reaction-type between the second and third seedings. Some strains, such as P.I. numbers 174563, 177824, 185790 and 190584, exhibited what may have been significant increases in resistance. On the other hand, several lines such as P.I. numbers 186474 and 186475 appeared to be a little more susceptible in the second seeding.

There was very little difference in reaction between the first and second seedings of most of the selections. No cases of appreciable increases in resistance were evident, but a few selections appeared to be more susceptible in the earliest seeding. Appler and P.I. 177824, for example, showed a fairly high degree of resistance in the second planting, but the older plants of the first planting were moderately or fully susceptible.

When these data were combined with those from other nurseries at Ames and Kanawha, Iowa, in 1953 and 1954, it was obvious that several selections had exhibited an outstanding degree of adult plant resistance. The best of these were P.I. numbers 174544, 174545, and 185783. The first 2 produced grain of good quality and were similar in general appearance. P.I. 174545, however, was at least a week later in maturity than P.I. 174544. P.I. 185783 produced very poor quality grain and obviously was poorly adapted to Iowa conditions. Other selections exhibiting almost as much adult resistance to crown rust as the 3 just mentioned included P.I. numbers 189628, 190584, 193040, 197278, and 197279. All of these produced grain of poor quality and were ill-suited to Iowa conditions.

Discussion.—This study emphasizes the unreliability of a single year's testing for adult reaction to crown rust. Many of the selections that were recorded as resistant in 1953 were obviously susceptible in 1954. These included both selections that showed a small number of large uredia and those that showed only flecking or small uredia in 1953. On the other hand, both of the above resistant classes contributed other selections in 1954 that were ranked as resistant and that produced no large uredia. These selections, of course, may or may not continue to exhibit resistance in future years. P.I. 174544 has been highly resistant in Iowa for 3 consecutive years. Shands (7) reported it as resistant in Wisconsin, and Ivanoff (4) reported it as resistant in Mississippi one year, but stated that it showed considerable susceptibility in the following year.

The reactions of the upper leaves in the greenhouse proved to be a severe test of field reaction, and all selections whose upper leaves were resistant under greenhouse conditions were highly resistant in the field in both 1953 and 1954. Greenhouse reactions in which the upper leaves were susceptible and the lower leaves resistant were less reliable. Some selections behaving in this manner in the greenhouse were resistant or moderately resistant in the field, while others were susceptible. Only one selection appeared to be completely susceptible in the greenhouse, yet showed a reasonably high degree of resistance in the field in 1954.

If a variety having adult plant resistance is to be used extensively in breeding, it would be desirable to test its reactions to different pure races of crown rust under controlled conditions. Results obtained indicate that certain types of adult resistance lend themselves to controlled greenhouse tests rather readily while others are unsuitable except under special treatment.

Field Crops Research Branch, A.R.S., U.S.D.A.,
and
Iowa Agricultural Experiment Station,
Ames, Iowa.

LITERATURE CITED

1. Earhart, R. W., and J. G. Moseman. 1953. Possible desirable germplasm in new oat introductions grown in Florida and North Carolina in 1952-53. U. S. Dept. Agr. Pl. Dis. Rptr. **37**: 597-598.
2. Finkner, R. E., R. E. Atkins, and H. C. Murphy. 1953. Inoculation techniques for crown rust of oats. Agron. Jour. **45**: 630-631.
3. Finkner, V. C. 1954. Genetic factors governing resistance and susceptibility of oats to Puccinia coronata Corda var. avenae, F. and L., race 57. Iowa Agr. Exp. Sta. Res. Bul. 411, p. 1040-1063.
4. Ivanoff, S. S. 1953. 1952 National Oat Newsletter (Sponsored by the National Oat Conference) **3**: 48-49.
5. Murphy, H. C. 1935. Physiologic specialization in Puccinia coronata avenae. U. S. Dept. Agr. Tech. Bul. 433, 48 p.
6. Peturson, B. 1944. Adult plant resistance of some oat varieties to physiologic races of crown rust. Canad. Jour. Res. Sec. C., **22**: 287-289.
7. Shands, H. L. 1952. 1951 National Oat Newsletter (Sponsored by the National Oat Conference) **2**: 58-59.
8. Simons, M. D. 1954. The relationship of temperature and stage of growth to the crown rust reaction of certain varieties of oats. Phytopathology **44**: 221-223.

DISEASE CYCLE OF SEPTORIA DISEASE OF OATS [1]

M. D. Huffman

SUMMARY

At least 2 types of spores were shown to play a role in the disease cycle. Microspores, previously not reported as occurring in the field, were shown to be a source of primary inoculum for the initial leaf lesions. The initiation of the black stem phase was shown to occur by water dissemination of the macroconidia formed in macropycnidia in the leaf lesions.

The disease caused by *Leptosphaeria avenaria* G. F. Weber has been known by at least 4 different names. Prior to Weber's[2] work, the disease was known merely as a leaf spot of oats. Because of the distinctive speckled appearance of the lesion following abundant pycnidial production, he proposed the name "speckled blotch." Following the description of the destructive culm-rotting phase,[3] the disease was known as "Septoria black stem" or "dark stem of oats." Since all of these phases are caused by the same organism, the disease is referred to as "Septoria disease of oats" in this paper.

Three types of fruiting bodies are known to exist in the life cycle of *L. avenaria*. The pycnidial stage, *Septoria avenae*, was described by Frank[4] in 1895; the perithecial stage, *Leptosphaeria avenaria*, by Weber[2] in 1921; and the micropycnidial stage by Johnson[5] in 1952.

The use of spore traps showed that microspores were disseminated in great abundance in June, indicating that the primary inoculum which causes the leaf spotting of oats at this time is produced principally in the form of microspores on overwintered oat stubble. From all indications the microspores were principally wind-borne, although it is possible that splashing rain may play a small role. It is possible that ascospores also may play a role in primary infection, although ascospores of Leptosphaeria were not collected on spore traps.

Approximately 2 weeks following the dispersal of inoculum, the leaf-spotting phase of the disease developed. It was generally more noticeable on the lower leaves since those were the leaves exposed at the time of the earliest spore dispersal. With inoculation spread over a long period of time the infection was nearly as heavy on upper leaves as on lower. The spots enlarged, often coalesced, and formed typically chocolate-brown to tan lesions, surrounded by flesh- to yellow-colored halos of necrotic tissue, blending into the green leaf tissue (Fig. 1). As the spots aged they became light tan to gray and the pycnidia developed throughout the lesions. These were the macropycnidia of *S. avenae* and their abundance depended somewhat upon temperature and moisture at the time they were formed. Very hot and dry weather retarded pycnidial development in the field.

Approximately 10-12 days after leaf spotting was noticed, lesions appeared on the leaf sheath at the ligule and at the interface of the sheath and culm surfaces (Fig. 2). These lesions constitute the initial stem infection which developed into the black-stem phase and resulted from inoculum originating in the macropycnidia borne in the leaf lesions. The macroconidia were exuded in a cirrhus through the ostiole of the pycnidium (Fig. 4), a process which makes them suited for dispersal by water, but not by wind.

[1] Accepted for publication January 10, 1955.
Journal Paper No. J-2647 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1176.

[2] Weber, G. F. 1922. Speckled blotch of oats. Phytopathology 12: 449-470.

[3] Meehan, Frances, and H. C. Murphy. 1949. Septoria avenae on oats in Iowa. (Abs.) Phytopathology 39: 15.

[4] Frank, B. 1895. Die neuen deutschen Getreidepilze. Deut. Bot. Gesell. Ber. 13: 61-65.

[5] Johnson, T. 1952. Cultural variability in Septoria avenae Frank. Canad. Jour. Bot. 30: 318-330.

Rain or heavy dew coming in contact with the spores in the water-soluble matrix washes them downward and into the leaf sheath, which provides a moist environment in which germination may occur, followed by infection of both the sheath and the culm.

Mycelium spreads from the point of sheath-culm infection. Intense darkening was seemingly favored by cool damp weather. As the fungus proceeds in growth, it may fill the hollow culm with mycelium (Fig. 3) and with favorable weather this stem rotting phase may cause very severe lodging.

At about the same period that culm infection is occurring, infection of the panicle and subsequently of nearly all above-ground parts may occur by the dispersal of macroconidia by splashing rain and by the mechanical contact of wet plants. Under some con-

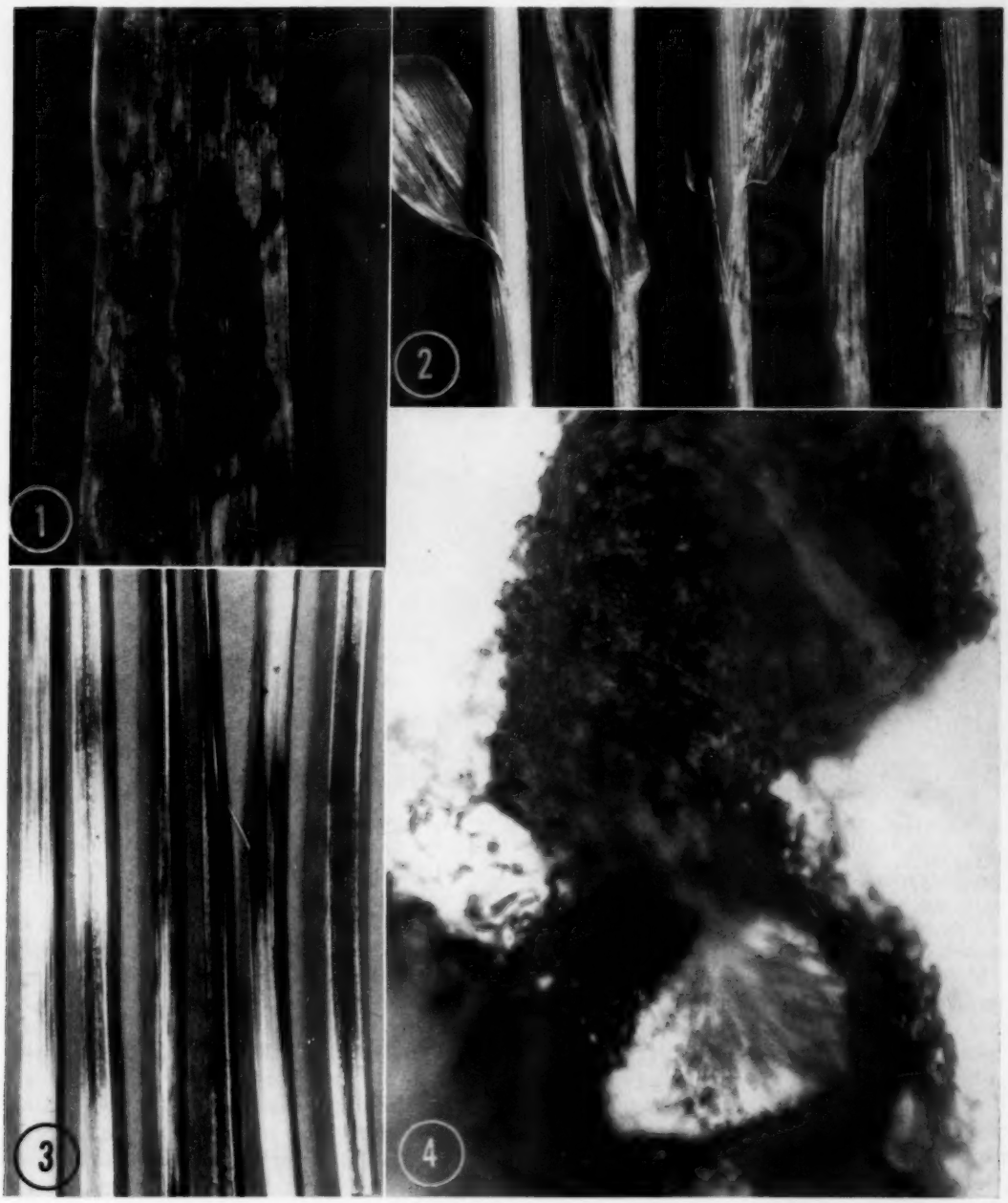


FIG. 1-4. Fig. 1. Leaf lesion showing scattered macropycnidia.—Fig. 2. Initial culm infection (×2).—Fig. 3. Culms split to show external lesions and mycelium filling the culm.—Fig. 4. Macropycnidium showing cirrhus of spores exuded through the ostiole (×480). Photographs by Dr. J. A. Browning.

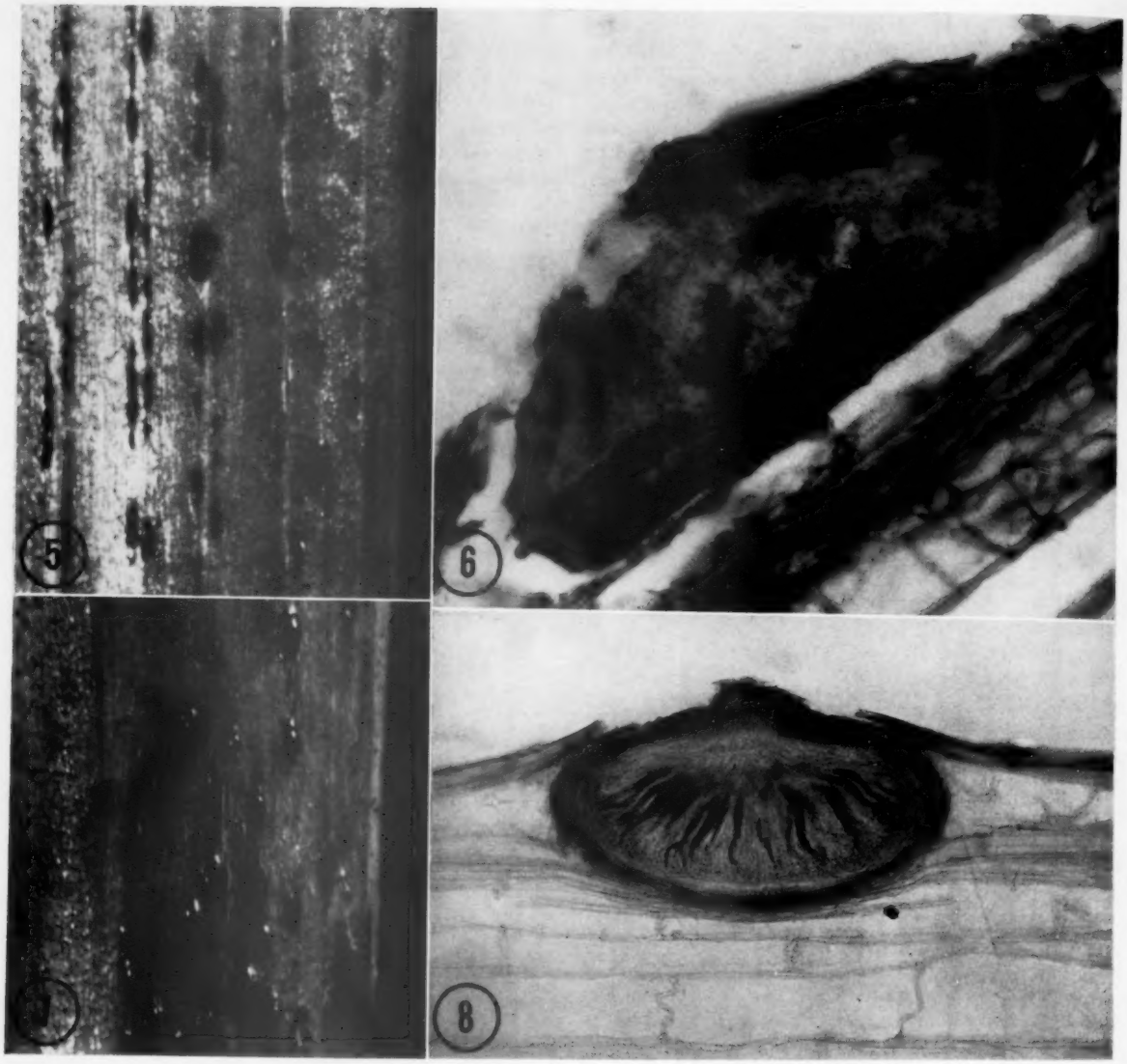


FIG. 5-8. Fig. 5. Micropycnidia on overwintered stubble (×25).—Fig. 6. Cross section of micropycnidium (×480).—Fig. 7. Perithecia on overwintered stubble (×25).—Fig. 8. Cross section of perithecium (×190). Photographs by Dr. J. A. Browning.

ditions the panicle infection results in the kernel blight phase of the disease in which the infected kernels become blackened, shrunken, and of low quality. Whether or not the mycelium present on and in the seed may cause a primary seedling infection is not as yet positively determined. The fact that no seedling infection has been seen in the field, plus the fact that such infection would certainly not be essential to the completion of the disease cycle, would tend to minimize the importance of this possibility.

Following harvest the infested stubble is a source of nutrient for the fungus, and it is in the mycelial form that the fungus persists through the late summer and winter. The appearance of micropycnidia (Fig. 5, 6) was noticed as early as the first week of April in every field visited. By the end of April and throughout May and June the micropycnidia were noticed in great abundance. About 2 weeks following the appearance of micropycnidia in the darkened lesions of the oat stubble, perithecia were formed (Fig. 7, 8) which developed mature ascospores by about the first week in June.

Thus inoculum for infection of the oat seedlings is provided from overwintered oat stubble and the disease cycle is completed.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY
IOWA STATE COLLEGE
AMES, IOWA

ACTIVITY OF AN ANTIVIRAL AGENT FROM NOCARDIA ON TWO VIRUSES IN INTACT PLANTS [1]

Reed A. Gray

SUMMARY

Leaf spray applications at a concentration of 1000 ppm of partially purified noformicin, an antibiotic produced by a species of *Nocardia*, markedly inhibited both the production of local lesions and systemic infections caused by southern bean mosaic virus and by tobacco mosaic virus in intact plants. Sprays containing 125 ppm of crystalline noformicin were equally effective. The antiviral activity of this antibiotic was similar to, but not as lasting as, that of thiouracil on the 2 viruses in bean and tobacco plants. Application of the antibiotic to roots of Pinto bean plants growing in sand reduced the number of local lesions produced in the primary leaves. Treatment of the viruses *in vitro* with the partially purified antibiotic at 1000 ppm and the pure noformicin hydrochloride at 250 ppm for 24 hours at 28°C or for 81 hours at 6°C did not reduce the activity of either virus.

Virus infections were inhibited when the sprays of the antibiotic were applied to the leaves starting 1 hour or 1 day after inoculation, as well as when the sprays were applied for several days before inoculation. Evidence was obtained that the antibiotic was translocated from the base of a bean leaf to the tip and in the reverse direction.

There have been several reports on the use of antibiotics against plant viruses diseases. Manil (6) was unable to prevent infection with tobacco mosaic virus by applying the antibiotics actinomycetin, actinomycin, penicillin, streptomycin, and tyrothricin. Beal and Jones (1) found no inhibition of tobacco mosaic or potato yellow dwarf viruses by aureomycin, chloromycetin, penicillin, streptomycin, or terramycin. Leben and Fulton (5) demonstrated an inhibitory effect of streptothricin and terramycin on lesion production by tobacco necrosis and tobacco ringspot viruses on detached leaves on agar plates. Terramycin also showed some activity against tobacco mosaic virus in detached leaves. Recently Schlegel and Rawlins (9) reported that an antibiotic from *Nocardia* inhibited production of tobacco mosaic virus in leaf discs floated on solutions of the antibiotic.

In the present studies the antiviral properties of this same antibiotic were studied on intact plants. The actinomycete producing the antibiotic was isolated by A. L. Pollard and S. Mayyasi of Knoxville, Tennessee, from an abandoned nest of African ants in an imported mahogany log. It is a member of the genus *Nocardia* and has been classified as *Nocardia formica* by Harris and Woodruff (4). The antiviral agent has been designated noformicin. Its production in culture and its crystallization have been described by Harris and Woodruff (4). Antiviral activity of noformicin in animals was reported by McClelland (7) who found that the antibiotic prolongs the life of mice infected with swine influenza virus or with the Lee and PR8 human influenza virus.

The work reported in this paper is concerned with the successful use of noformicin in inhibiting the production of local lesions and systemic infections by southern bean mosaic virus and by tobacco mosaic virus in intact plants of bean and tobacco, respectively. Thiouracil, which was reported to inhibit tobacco mosaic virus biosynthesis by Commoner and Mercer (2, 3) and Nichols (8), was tested on intact plants for comparison. It is believed that this is the first successful use of an antibiotic against virus diseases in intact plants.

MATERIALS AND METHODS.—In most cases, plants were grown in light soil in the greenhouse at a constant temperature of 26°C. Where the antibiotic was applied through the roots, the plants were grown in washed river sand and watered with Hoagland's nutrient solution. The initial inoculum of southern bean mosaic virus was kindly supplied by Dr. W. J. Zaumeyer and the purified common tobacco mosaic virus by Dr. J. Bonner and Dr. S. Wildman.

The antibiotic was supplied by Dr. H. M. Shafer, Research Laboratories, Chemical Division, Merck & Co., Inc. Unless indicated otherwise, it was approximately 10 per cent pure, based on the survival index of mice infected with swine influenza virus. Therefore, the aqueous spray solutions containing 1000 ppm by weight of the crude antiviral agent actually contained approximately 100 ppm of the pure material. The impurity was mostly calcium chloride, which was found to have no inhibitory effect on lesion production by southern bean mosaic virus. A pure sample of crystalline noformicin hydrochloride was available in small quantities and was used in some of the later experiments.

Twin primary leaves of 4 Idaho Pinto bean plants were inoculated with a suitable dilution of southern bean mosaic virus when they were almost fully expanded, about 10-14 days after planting. A suspension of 1 g of virus-infected Stringless Green Refugee bean leaves in 500 ml of water was used for inoculation. Leaves were dusted with carborundum and rubbed uniformly with the virus suspension. The leaves were rinsed a few minutes after inoculation and blotted dry. Later, 1 of the twin primary leaves of each plant was sprayed on the top and bottom surfaces with 1000 ppm of the crude antibiotic; the other twin primary

[1] Accepted for publication January 25, 1955.

leaf of each plant was sprayed with distilled water and served as a control.

In the experiments on systemic infections, Stringless Green Refugee and Bountiful beans were used. One primary leaf of each plant was inoculated. Some plants were then sprayed with the antibiotic on all leaves, whereas the control plants were sprayed with distilled water. After 1 or 2 weeks, the trifoliate leaves of treated plants were harvested, washed, blotted dry, weighed, and homogenized in 10 times their weight of distilled water in an ice-cooled Servall Omni-mixer for 1 minute. The juice was squeezed through cheesecloth and rubbed onto 1 twin primary leaf of each of 4 Pinto bean plants as described. Juice of the control plant was extracted in exactly the same manner and rubbed onto the other twin primary leaves of the same bean plants. The lesions were counted after 6 days.

The effect of noformicin on the production of local lesions by tobacco mosaic virus in plants of *Nicotiana rustica* L. was determined by a procedure similar to that used with bean plants. The procedure differed in that after uniform inoculation of all leaves on a plant, the left half of each leaf was sprayed with the antibiotic, while the right half was shielded with a paper towel.

For systemic infection by tobacco mosaic virus, plants of *Nicotiana tabacum* L. var. White Burley, approximately 1 ft. high, were inoculated on the eighth leaf from the top. The leaf was rinsed after 5 minutes, and all the leaves of 1 of each pair of plants were sprayed at intervals with 1000 ppm of the crude antibiotic. The other plant of each pair was sprayed with water. One week after inoculation, every other leaf above the inoculated leaf was harvested, weighed, and the virus extracted as described above for the bean virus, except that 0.1 M phosphate buffer (pH 7.0) was used instead of distilled water. For assaying, this extract was rubbed on the left halves of the leaves of plants of *N. rustica* and that from the untreated plant on the right halves.

RESULTS.—*Effect of noformicin on local lesion production by southern bean mosaic virus.*—In 2 experiments, spraying bean leaves 1 hour after inoculation and again on each of the 2 following days with 1000 ppm of the crude noformicin almost completely inhibited the formation of lesions within 4 days after inoculation (Table 1). By 7 days after inoculation, the inhibition was not quite so marked (79 per cent). In all 3 experiments reported in Table 1, noformicin at 1000 ppm greatly delayed development of local lesions and markedly reduced the final number of lesions produced. In a parallel experiment, thiouracil at 100 ppm spray reduced the number of lesions 93 per cent at 4 days after inoculation and no more lesions developed on the thiouracil treated leaves by 7 days after inoculation. The data show that either the effect of noformicin is not permanent or that noformicin affects only the rate of lesion formation. On the other hand, the effect of thiouracil appeared to be more lasting. Noformicin at 1000 ppm caused no visible injury to the bean plants, whereas thiouracil at both 100 ppm and 50 ppm caused chlorosis and stunting in the young leaves.

TABLE 1.—*Activity of crude noformicin and thiouracil on local lesion production by southern bean mosaic virus*

Exp. No.	Treatment	Average number of lesions on 4 leaves of 4 plants	
		4 days after inoculation	7 days after inoculation
1	Noformicin, 1000 ppm	0	48
	Control	220	235
2	Noformicin, 1000 ppm	2	86
	Control	288	407
	Thiouracil, 100 ppm	16	16
	Control	224	384
	Thiouracil, 50 ppm	28	38
	Control	285	379
		5 days after inoculation	
3	Noformicin, 1000 ppm	21	56
	Control	190	189
	Thiouracil, 100 ppm	15	19
	Control	114	114

Effect of noformicin on local lesion production by tobacco mosaic virus.—When the top and bottom surfaces of the left halves of 7 leaves of *N. rustica* were sprayed with 1000 ppm of the crude noformicin 1 hour after inoculation and again on each of the 2 following days, the average number of lesions that developed on the sprayed halves was 14 three days after inoculation, whereas the average for untreated halves was 96. Six days after inoculation, there was an average of 91 lesions on the controls and 66 on the treated leaf-halves. When the left sides of 7 leaves were sprayed with 100 ppm thiouracil in the same manner, an average of 8 lesions had developed on the sprayed halves 3 days after inoculation, whereas an average of 74 had developed on the control halves. The average lesion counts were 11 for the thiouracil-treated halves and 85 for the control halves at 7 days after inoculation. Inhibition of lesion formation with noformicin again was not permanent. Either the virus was not inactivated by the antibiotic and resumed reproduction when the concentration of the antibiotic diminished, or the antibiotic merely reduced the rate of virus multiplication. The inhibiting effect of thiouracil was more permanent, but it stunted the plants. Noformicin caused no visible damage to the plants at the concentration used.

In another experiment the left halves of each leaf on 2 plants of *N. rustica* were sprayed 4 times with 1000 ppm noformicin, once 1 hour after inoculation and once on each of the 3 following days. On the fourth day after inoculation the average number of lesions on the 10 treated half-leaves was 26 and on the untreated leaves 169. On the seventh day the averages were 65 and 169, respectively. This represents an 85 per cent inhibition at 4 days and 62 per cent inhibition at 7 days. It appears that 4 sprays gave

more lasting protection than did 3 sprays. Lesions in both experiments reported above were counted on the first and fourth days after the last spray application.

Effect of the antibiotic on systemic infection by tobacco mosaic virus.—Four sprays of 1000 ppm of the crude antibiotic were applied to 3 plants of *Nicotiana tabacum* var. White Burley 1 hour after inoculation and once each on each of the next 3 days. Three other plants were sprayed with distilled water at the same times. Noninoculated leaves were harvested 1 week after inoculation and the juice was extracted from the treated and untreated plants. The 2 juices were rubbed on the opposite halves of *N. rustica* for assaying as previously described. The ratios of average number of lesions produced on 6 half-leaves by extracts from treated plants to that produced by extracts from the controls were 0.9/411, 58/498 and 4.6/496 in the 3 pairs of plants tested. The antibiotic markedly inhibited systemic production of tobacco mosaic virus. Vein-clearing symptoms of systemic infection were evident after 1 week in the young leaves of all 3 control plants, but not in the antibiotic-treated plants. Two weeks after inoculation the 4 even numbered leaves of each of these same plants were tested. The ratios were then 188/331, 297/353 and 152/305, respectively. Evidently the marked inhibition caused by the antibiotic the first week was not permanent, and virus multiplication resumed after the spraying with the antibiotic was stopped.

In another experiment, 7 sprays of 1000 ppm of the crude noformicin markedly inhibited the virus production over a period of 2 weeks. The assays of the extracted juice from a treated tobacco plant showed 89 per cent fewer lesions than did the control. In the same experiment 4 sprays of 100 ppm of thiouracil inhibited systemic infection by tobacco mosaic virus to a slightly less extent. Assays of the extracted juice from the thiouracil-treated plant showed 82 per cent fewer lesions than did the control. No more sprays of thiouracil were applied after the fourth because the plant was being stunted by it.

Effect of the antibiotic on systemic infection by southern bean mosaic virus.—In 2 young Refugee bean plants, 6 sprays of the crude antibiotic at 1000 ppm applied during the first week after inoculation caused no significant reduction in southern bean mosaic virus production in noninoculated leaves. Assays for virus were made 2 weeks after inoculation.

When tested under the same conditions, except that only 4 sprays were applied, thiouracil at 100 ppm inhibited virus production. In the assays of juices from 2 pairs of systemically infected plants, the ratios of the average number of lesions produced by extracts from treated plants to that produced by the controls were 31/201 and 123/216. Thiouracil retarded the growth of the young leaves.

In Bountiful bean plants at an older stage of growth, noformicin inhibited the systemic production of the virus considerably. In tests with 2 pairs of infected plants, the ratios of the average number of local lesions produced from extracts of the treated plants to that produced by the controls were 11/72 and 9/29. The experiment was carried out in the same way as described above for Refugee plants.

Absorption of the antibiotic through the roots and its effect on the bean virus.—Evidence that the antibiotic was absorbed by the roots and translocated to the leaves was found when 1000 ppm of the crude antibiotic in Hoagland's nutrient was applied to the roots of Pinto beans growing in sand. The antibiotic solution was applied over a period of 1 week. Four days after the first treatment, the primary and first trifoliate leaves of 4 treated plants and 4 control plants were inoculated with the southern bean mosaic virus. Six days later the lesions were counted. The antibiotic inhibited the production of local lesions markedly in the primary leaves but it had no appreciable effect on virus formation in the trifoliate leaves. The average number of lesions on 8 primary leaves of plants treated with noformicin through the roots was 64 and on 8 leaves of untreated plants the average number was 212. This represents a 77 per cent reduction in lesions. In the 12 trifoliate leaflets of treated plants the average number of lesions was 133 and on 12 trifoliate leaflets of control plants the average was 146. No harmful effects of the antibiotic on the plants was detected.

Activity of pure crystalline noformicin.—A pure preparation of the antibiotic was obtained as the crystalline hydrochloride. The activity of the pure preparation against local lesion production by southern bean mosaic virus was compared with that of the crude material which was used in all previous experiments reported in this paper. The antibiotic in each case was applied 1 hour after inoculation and again on the next 2 days. Four primary leaves of 4 different Pinto bean plants were sprayed with each level of antibiotic and the 4 untreated twin leaves served as controls. On a weight basis the pure noformicin hydrochloride was over 5 times as active against the virus as the crude product (Table 2). The crude noformicin at 2000 ppm and the pure noformicin hydrochloride at 400 ppm both caused slight burning

TABLE 2.—*Activity of pure crystalline noformicin compared with that of crude noformicin against local-lesion production by southern bean mosaic virus*

Treatment	Concentration ppm	Percentage reduction in no. of lesions 4 days after inoculation	Percentage reduction in no. of lesions 7 days after inoculation
Pure noformicin · HCl	400	99	86
	200	100	70
	100	95	47
	50	55	15
Crude noformicin	2000	100	94
	1000	99	79
	500	83	30
	250	80	14

of the leaves, but no injury was noticed at the lower concentrations used.

In another experiment, pure noformicin hydrochloride at both 500 ppm and 250 ppm caused complete inhibition of lesion development for 7 days after inoculation. At 125 ppm it caused 77.5 per cent reduction in the number of lesions 7 days after inoculation.

Effect of the time interval between inoculation and spraying.—In most of the previous experiments the first spray with the antibiotic was applied 1 hour after inoculation. When the first spray with the antibiotic was applied 16 hours or 1 day after inoculation, it still was very effective in inhibiting local lesion production by southern bean mosaic virus. A spray containing 200 ppm of crystalline noformicin was first applied 16 hours after the inoculation of Pinto bean leaves with southern bean mosaic virus. The leaves were sprayed again on the next 2 days. The ratio of the average number of lesions produced on 4 treated primary leaves of 4 plants to that on the 4 untreated twin leaves of the same plants was 1.5/189 when lesions were counted 4 days after inoculation. When counts were made 7 days after inoculation, the ratio was 13/192.

When the first of 3 treatments with crude noformicin at 1000 ppm was applied 24 hours after inoculation, the ratio of the average number of lesions on treated leaves to that on untreated leaves was 1/181 at 4 days and 41/287 at 7 days after inoculation. Besides greatly reducing the number of lesions, the antibiotic also reduced the size of the lesions. Applying the antiviral agent before inoculation also delayed lesion development and reduced the size of the lesions in Pinto bean plants.

When the first sprays of the antibiotic were applied 1 or 2 days after inoculation of 2 White Burley tobacco plants with tobacco mosaic virus, little or no effect on systemic infection was found. However, 3 sprays of 1000 ppm of the crude noformicin applied on 3 successive days to tobacco plants which were inoculated on the fourth day prevented systemic infection in 1 plant for at least a week. When more leaves of the same treated and control plant were tested 2 weeks after inoculation, the ratio of the average number of lesions produced by an extract of the treated plant on 7 half-leaves of *N. rustica* to that by an extract of an untreated plant was 40/114. When extracts from another pair of plants were tested on 12 half-leaves 1 week after inoculation, the lesion ratio was 15/116.

In another experiment southern bean moasic virus was inoculated into the upper surfaces of Pinto bean leaves and 1000 ppm of the crude antibiotic was sprayed only on the lower surfaces. The treated leaves had no lesions when counted 4 days after inoculation while the average of the control leaves was 282. Three days later the average lesion counts for the treated and untreated leaves were 192 and 287, respectively. These results indicate that the antibiotic was absorbed by the leaves and that it did not have to enter the punctures made by the carborundum in order to be effective.

Translocation of noformicin.—A solution of crystalline noformicin hydrochloride was sprayed on the primary leaves of Pinto beans at a high concentration of 500 ppm. Two days later, the trifoliate leaves were inoculated with southern bean mosaic virus. On the 2 following days the primary leaves again were sprayed with the antibiotic. During the spraying, the trifoliate leaves were shielded with a paper bag. The average number of lesions on 12 nonsprayed trifoliate leaflets of the treated plants was 80 and the average on those of the untreated plants was 134. This represents a 40 per cent reduction in the number of lesions on the nonsprayed leaves of the treated plants. There might have been some translocation of the antibiotic from the older primary leaves to the young trifoliate leaves. However, the noformicin sprays caused considerable injury to the sprayed leaves, and this may have influenced the young leaves, thus causing a reduction in number of lesions.

In another experiment, 500 ppm of the crystalline noformicin was applied to the basal half of 1 of the twin primary leaves on each of 4 Pinto bean plants after all leaves had been inoculated with southern bean mosaic virus. Four fine sprays were applied on different days until droplets formed. During the spraying, the apical half of the leaf was shielded with a paper towel. Only 19 per cent as many lesions developed on the nonsprayed halves as developed on comparable areas of untreated leaves. In a similar manner treating the apical half of the leaf caused 68 per cent reduction in the number of lesions produced on the basal half. These results indicate that the antibiotic was translocated from the base of the leaf toward the tip and in the reverse direction to a lesser extent.

Effect of treating plant viruses in vitro with noformicin.—Southern bean mosaic virus was incubated at 28°C for 24 hours and at 6°C for 81 hours in solutions containing 250 ppm of pure noformicin hydrochloride. Solutions of the virus at the same dilution but without any antibiotic were incubated at the same temperatures and served as controls. Samples of the treated virus and of the controls were removed after 6, 24, and 81 hours and used for the inoculation of 4 twin primary leaves of Pinto beans. The inoculum was washed off the leaves 3 minutes after application. No reduction in the number of local lesions was obtained by treating the virus *in vitro* for as long as 24 hours at 28°C or 81 hours at 6°C. Similar results were obtained with the crude antibiotic at 1000 ppm.

In like manner it was shown that tobacco mosaic virus was not inactivated by treatment with the antibiotic *in vitro*. These findings are similar to those of Harris and Woodruff (4) who reported that animal viruses were not inactivated by incubation *in vitro* with the antibiotic.

DISCUSSION.—Although noformicin was found to

delay and to inhibit markedly the reproduction of southern bean mosaic virus and of tobacco mosaic virus, it did not destroy the viruses. Virus multiplication apparently resumed after spray applications of the antibiotic were stopped. The *in vitro* experiments also showed that noformicin did not inactivate the viruses, indicating that the antibiotic interfered with the reproduction of the virus in the plant tissue.

The 2 viruses used in this study are very stable, and it seems possible that other less stable viruses might be inactivated if their reproduction were inhibited for 1 week. Therefore, noformicin might be useful against plant viruses that are less stable than southern bean mosaic and tobacco mosaic virus.

The successful inhibition of 2 plant virus infections in intact plants by spray applications of the antibiotic noformicin are encouraging from another standpoint. It indicates that there is some hope of finding other plant antiviral agents among the products produced by microorganisms.

RESEARCH LABORATORIES
CHEMICAL DIVISION
MERCK & CO., INC.
RAHWAY, NEW JERSEY

LITERATURE CITED

1. BEALE, HELEN PURDY, AND C. R. JONES. 1951. Virus disease of tobacco mosaic and potato yellow dwarf not controlled by certain purified antibiotics. Boyce Thompson Inst. Contr. **16:** 395-407.
2. COMMONER, B., AND F. L. MERCER. 1951. Inhibition of the biosynthesis of tobacco mosaic virus by thiouracil. Nature [London] **169:** 113-114.
3. COMMONER, B., AND F. L. MERCER. 1952. The effect of thiouracil on rate of tobacco mosaic virus biosynthesis. Arch. Biochem. and Biophys. **35:** 278-289.
4. HARRIS, D. A., AND H. B. WOODRUFF. 1953. A crystalline antiviral agent produced by a new species of Nocardia. I. Cultural characteristics, fermentation studies, and production of the antiviral agent. In H. Welch and F. Martí-Ibáñez, ed. Antibiotics annual 1953-1954. Medical Encyclopedia Inc., New York. p. 609-614.
5. LEBEN, C., AND R. W. FULTON. 1952. Effect of certain antibiotics on lesion production by two plant viruses. Phytopathology **42:** 331-335.
6. MANIL, P. 1947. Action negative, sur le virus de la mosaique du tabac, de la penicilline et du prontosil. Ann. Inst. Pasteur **73:** 294.
7. MCCLELLAND, LAURELLA. 1953. A crystalline antiviral agent produced by a new species of Nocardia. II. Antiviral activity in animals. In H. Welch and F. Martí-Ibáñez, ed. Antibiotics annual 1953-1954. Medical Encyclopedia Inc., New York. p. 615-621.
8. NICHOLS, C. W. 1953. Thiouracil inhibition of tobacco mosaic virus multiplication. Phytopathology **43:** 555-557.
9. SCHLEGEL, D. E., AND T. E. RAWLINS. 1954. Inhibition of tobacco mosaic virus by an antibiotic from an actinomycete, Nocardia species. Phytopathology **44:** 328-329.

TIME OF PEACH TWIG INFECTION BY XANTHOMONAS PRUNI IN RELATION TO SPRING-CANKER INCIDENCE [1]

Angelos V. Adam, Dwight Powell, and H. W. Anderson[2]

SUMMARY

Studies over a 2-year period have shown that August is the optimum time in Illinois for the experimental application of *Xanthomonas pruni* (E. F. Sm.) Dowson to peach trees for high incidence of twig-canker the following spring. Spring-canker incidence is in direct proportion to the amount of the previous summer's foliage infection. Bacteria will survive the winter in peach twig tissues inoculated by the "forced-stream" method during the latter part of the growing season. Bacteria introduced into the cortical tissue of the current year's twig can survive the winter and produce typical spring-cankers. From inoculations it is evident that the terminal portion of the twig is the most suitable tissue for infections that survive the winter and produce inoculum the following spring. However, the ratio of terminal ("black-tip") to internodal spring-cankers over the period of this study was approximately 1:1.

One of the most important aspects of the perpetuation of the bacterial spot disease of peach has been the exact time and duration of the period in which the bacteria responsible for spring-canker formation and subsequent primary foliar infection enter the host shoot tissue. With a better knowledge of this period it is possible that a more intelligent approach could be made in applying control measures.

Direct proof of the period when twig tissue is most susceptible to infection would be difficult to achieve from observation on natural infections. Therefore, the problem was approached by applying experimental in-

[1] Accepted for publication January 28, 1955.

[2] Respectively, Assistant in Plant Pathology, Professor in Plant Pathology, and Professor in Plant Pathology, Emeritus; Division of Plant Pathology, Department of Horticulture, University of Illinois and Illinois Agricultural Experiment Station, Urbana, Illinois.

oculations to simulate natural infections. These inoculations and the infections that developed from them were studied as a means of ascertaining the most probable time of twig infection in relation to the survival of bacteria that provide the primary spring inoculum for foliar infections.

MATERIALS AND METHODS.—Inoculum was prepared from young bacterial cells subcultured in modified Emerson broth consisting of 1 g yeast extract, 4 g Bacto-peptone, 4 g beef-extract, 2.5 g sodium chloride, 10 g Cerelose (technical grade glucose), and 1000 ml distilled water. One hundred fifty ml of Emerson broth in each of several 500-ml flasks were seeded with a liberal amount of bacteria taken from a 48-hour-old Emerson-agar slant culture. The flasks were then placed on a rotary shaker (250 rpm and 2 in. radius) in order to permit more rapid growth of the bacteria. After 30 hours of shaking, the yellowish bacterial suspension was diluted at the ratio of 1000 ml to 6 gal. of tap-water, poured into a power-sprayer tank, and applied to peach trees ("forced-stream method"). The spray was applied with a single nozzle gun with a 3/64 in. aperture adjusted to a solid stream at 200 lb. pressure. During the inoculations the spray gun was kept at a distance of approximately 4-6 ft. from the tree to avoid leaf injury and was pointed upward at an approximately 60 degree angle so that the stream would contact the underside of the leaves as much as possible. All inoculations were made between 10 a.m. and 2 p.m. during periods of sunshine, high temperatures, and with as little wind as possible (3). A block of 120 vigorously growing 2-year-old Elberta peach trees was divided into single-tree plots with each plot replicated 10 times.

In 1952, "needle-puncture" inoculations were made on 2-year-old Elberta peach trees arranged in single-tree plots. Inoculum was obtained from 48-hour-old Emerson agar slants. An incision approximately 1 cm long was made in the bark of the twig and a liberal amount of inoculum was transferred to the cortical tissue. The incision was then closed by gentle pressure applied by the thumb and fore-finger on the bark along the longitudinal axis of the twig. In this manner, 35 twigs were inoculated on each tree with 5 inoculations per twig extending from the tip to the seventh node. The first tree was so treated August 9; the second, August 22; the third, September 3; the fourth, September 18; the fifth, October 5; and the sixth, October 24. A check tree for each inoculated tree was established by making the incision without the inoculation. Beginning November 1, a few days after the last inoculation, attempts were made to isolate the organism at weekly intervals from all "needle-puncture"-inoculated trees. To secure isolations after December 15, it was necessary first to expose the inoculated twigs to a temperature of 75° F, 90 per cent relative humidity, and continuous illumination for a 5-day period in the greenhouse.

Trees inoculated with the "forced-stream" method were indexed twice. The percentage of infected leaves was determined immediately following the appearance of the symptoms on the foliage approximately 10 days after inoculation. The next spring, the number of cankers which had developed was recorded.

RESULTS.—Artificial inoculation of the foliage by the "forced-stream" method was most successful during August (Table 1). The August inoculations not only produced a higher percentage of infected leaves but also resulted in more spring-cankers. Weekly isolations beginning on November 1 from inoculations made on August 9 and 22, September 3 and 18, and October 5 and 24, yielded the following percentages of successful isolations: 81.64, 67.50, 44.89, 41.36, 6.42, and 2.34, respectively. Thus, the highest percentage of positive isolations was obtained from the August 9 and the lowest from the October 24 inoculations. All isolations from the check trees were negative. From these same isolations, tissue taken from the first, second, third, fifth, and sixth internodal areas produced the following percentage of positive isolations: 92, 78, 74, 38, and 18, respectively. In this case the first internode was at the tip of the twig. According to these data, the tip end is the most susceptible part of the twig, and provides optimum conditions for the overwintering of the bacteria.

DISCUSSION.—Spring-canker development is, in general, in proportion to previous summer foliage infection. However, some plots, for example the one inoculated July 3, developed a relatively low foliage infection but responded significantly in canker formation (Table 1). One explanation of this is that the organism could reach the cortical tissues of the twig under certain climatic conditions when foliage infection could not occur. It is also possible that a mild foliage infection early in the season could supply sufficient inoculum for an August invasion of the twigs. This is supported by the fact that in this test a larger number of spring-cankers developed from the earlier inoculations than from the later ones although there was insignificant foliage infection in both instances.

TABLE 1.—*Extent of foliage infection by X. pruni 10 days following inoculation and spring-canker incidence the following spring*

Date inoculated in 1952 and 1953	Percentage of foliar infection		Average number of spring-cankers per tree[a]	
	1952	1953	1953	1954
May 9		3.6		2.33
June 6		4.3		5.67
July 3	1.1	7.7	2.72	8.00
August 4	1.5	10.7	6.33	22.33
August 8	80.1		20.67	
August 20		74.3		9.67
September 8	1.6	5.6	8.50	6.00
September 26	0	0	5.11	3.67
October 18	0	0	3.50	1.00
November 6		0		0.33
Noninoculated check			0.04	0.67
L.S.D. 5% level	4.75	4.83	3.59	3.50

[a] Each figure is the average of counts on 10 trees.

It is conceivable that inocula supplied by both natural and artificial means contributed to the formation of the cankers. Unfortunately, in studies of this kind it is necessary to utilize artificial inoculation which distributes a more concentrated mass of bacteria than is probable in nature. Under natural conditions in Illinois, as many as 5 spring-cankers for a 2-year-old tree would be a very high rate of survival. Yet by experimental inoculation, an average of 22.33 cankers per tree (Table 1, August 4, 1954) was obtained in 1 case. From such results it is evident that canker incidence stemming from natural inoculations before and after the month of August would be extremely low.

There has been much discussion in the literature (1, 2, 5) concerning the relative importance and abundance of the "black-tip" and the internodal types of cankers. During the course of this experiment when hundreds of cankers were observed, it was noted that 61 and 54 per cent of the spring-cankers observed in 1953 and 1954, respectively, were of the "black-tip" type. These are distinct from terminal die-backs produced from other causes in that the latter show a definite line of demarcation between the dead and living tissue when growth is resumed in the spring. During the time of this work very few nonbacterial terminal die-backs occurred in the noninoculated check trees. This would indicate that in most cases where cankers did occur on the inoculated trees, infections by *X. pruni* were responsible.

The greater resistance to invasion of the older or basal portion of the twig probably is in part due to the fact that in the basal portion, the stomata are older, less active, and possibly fewer per unit area than are those in the apical area. Also, the presence of a phellogen layer beneath the epidermis in the basal portion may wall off the cortex against external invasion. The resistance of the overwintered twigs to invasion, even when the cortex is punctured, might be explained by the compact character of the cortical cells.

"Needle-puncture" inoculations showed that *X. pruni*, if introduced into the cortical tissue of the current year's twig during August, September, or October, can survive in the twigs and produce typical spring-cankers the following spring. Shortly after inoculation the host forms a corky layer of cells and walls off the infected area. If invasion occurs outside of this layer, because of extreme activity of the bacteria, a second layer of cells forms, and this process is repeated until the bacterial areas are successfully isolated and to a certain extent are protected from adverse environmental conditions. In the early spring before the host cells start division, the masses of bacteria renew their activity and invade the cortical region by the formation of zoogloeal masses which induce spring-canker formation.

When weekly isolations were made from the "needle-punctures," positive isolations were high from November 1 until the middle of December. After this period, no bacteria could be isolated directly when the twigs were brought into the laboratory. Cold winter temperatures had so reduced or inactivated them that they were difficult to find. When twigs collected after December 15 were exposed to 5 days of continuous warm temperatures (about 75°F) and a high relative humidity, the number of successful isolations greatly increased. This indicates that a sufficient number of bacteria are able to survive the winter months in the cortical tissues of the twig to cause cankers in the spring.

Division of Plant Pathology, Department of Horticulture
University of Illinois and Illinois Agricultural Experiment Station
Urbana, Illinois

LITERATURE CITED

1. Adam, A. V., H. W. Anderson, and D. Powell. 1953. Origin of peach bacterial spot spring-cankers. (Abs.) Phytopathology **43**: 465.
2. Anderson, H. W. 1953. "Spring-cankers" (Xanthomonas pruni) or "black tip"? of peach trees. U. S. Dept. Agr. Pl. Dis. Rptr. **37**: 16-17.
3. Anderson, H. W., and D. Powell. 1950. Relation of time of day and other factors in mass inoculation with Xanthomonas pruni. (Abs.) Phytopathology **40**: 1.
4. Dunegan, J. C. 1932. The bacterial spot disease of the peach and other stone fruits. U. S. Dept. Agr. Tech. Bul. 273, 54 p.
5. Goldsworth, M. C., and R. A. Wilson. 1952. Terminal bud and shoot infections and the overwintering of Xanthomonas pruni in peach trees. U. S. Dept. Agr. Pl. Dis. Rptr. **36**: 408-409.
6. Larsh, H. W., and H. W. Anderson. 1948. Bacterial spot of stone fruits with special reference to epiphytotics and dissemination of the causal organism. Ill. Agr. Exp. Sta. Bul. 530, 55 p.
7. Thornberry, H. H., and H. W. Anderson. 1941. Overwintering of bacterial spot of peach. U. S. Dept. Agr. Pl. Dis. Rptr. **25**: 268-269.

Antagonistic Action of Helminthosporium sativum and Gibberella zeae to Infection by Tilletia spp. Causing Bunt of Wheat.[1] HARRY W. SCHROEDER. Observations in the spring wheat disease garden at St. Paul, Minnesota, have indicated that infesting wheat seed with both root–rotting and bunt–causing fungi may result in failure to obtain good infections with the latter. These observations have been substantiated by experimental work.

Seed of 2 varieties of wheat, Ulka C. I. 11478 and Newthatch C. I. 12318, were infested concomitantly with spores of bunt fungi and with root–rotting organisms in preliminary greenhouse tests in the winter of 1952–53. All of the seed was dusted with spores of *Tilletia* spp. before it was planted in 6-in. pots of autoclaved soil. The contents of 1 pot of each variety received no other fungus inoculum. The soil in a second pot was infested, at planting time, with inoculum of *Helminthosporium sativum* Pam., King & Bakke grown on a mixture of wheat and barley grain. The third pot received a similarly prepared inoculum of *Gibberella zeae* (Schw.) Petch; and the fourth pot received a mixture of both inocula. Bunted heads resulted only in those pots where the bunt inoculum alone had been used. This evidence warranted further tests in the field.

Tilletia–infested seed of Ulka wheat was planted in 6–ft. rows at St. Paul, Minnesota on May 5, 1953. At planting time, a mixture of inocula of *H. sativum* and *G. zeae* grown on the wheat–barley mixture was spread in alternate seeded-rows. The number of heads produced and the percentage infected with bunt were determinated for each row at plant maturity (Table 1). The average reduction in amount of bunt in rows supplied with inoculum of root–rotting fungi was 51 per cent.

In 1954, a more extensive field planting of 3 varieties of wheat was sown in a modified split–plot design to minimize the possibility of the root-rotting organisms growing from a row of inoculated plants to an adjacent row of non-inoculated ones. Each variety was planted in 6–ft. rows, replicated 3 times in a section of the plot containing noninoculated plants and 3 times in a section containing plants inoculated with both root–rotting fungi. Other procedures were the same as those used in the previous experiment. The presence of root–rot inoculum reduced the amount of bunt infection in the heads of mature plants by 78 per cent in the variety II-46-13 C. I. 13030, by 64 per cent in Ulka, and by 61 per cent in Lee C. I. 12488 (Table 2).

TABLE 1.—*Percentage of bunt in the wheat variety Ulka in 1953 at St. Paul, Minnesota, in plants grown from seed infested with Tilletia spp. with and without concomitant infestation with Helminthosporium sativum and Gibberella zeae. Average of 4 replications*

Treatment	No. of heads	Percentage bunt
Not infested with root–rotting fungi	193	49
Infested with root–rotting fungi	181	24

[1] Paper No. 3223, Scientific Journal Series, Minnesota Agricultural Experiment Station.

TABLE 2.—*Percentage of bunt in 3 varieties of wheat in 1954 at St. Paul, Minnesota, in plants grown from bunt–infested seed with and without simultaneous inoculation with Helminthosporium sativum and Gibberella zeae. Average of 3 replications*

Variety	Not inoc. with root rotting fungi		Inoc. with root rotting fungi		Percentage reduction in amount of bunt
	No. of heads	Percentage bunt	No. of heads	Percentage bunt	
II-46-13	275	46	227	10	78
Ulka	223	85	194	41	64
Lee	294	41	202	16	61

Some antagonisms apparently exist between the organisms causing bunt of wheat and those causing root rot. Whether this is due to antibiotic substances or to competition for an infection court has not been determined. In the light of these results, however, it might be well to re-examine the temperature relations of infection by the bunt fungus. The increased activity of soil microorganisms at higher temperatures may be a factor in the decreased infectivity of bunt at such temperatures.

These results point out the inadvisability of attempting to obtain bunt resistance ratings and root–rot resistant ratings simultaneously.—Department of Plant Pathology, Institute of Agriculture, St. Paul, Minnesota.

Calcium Deficiency and Infection of Nicotiana glutinosa by Tobacco Mosaic Virus.[1] M. CHESSIN AND H. A. SCOTT. Studies on the relation between host mineral nutrition and plant virus infection in local lesion hosts generally have dealt with variations in nitrogen, phosphorous, and potassium supplies[2]. This

[1] Based in part on a thesis submitted by the junior author to the Graduate School of Montana State University in partial fulfillment of the requirements for the degree of Master of Arts. This investigation was supported by research grant G 4111 from the U. S. Public Health Service.

[2] Bawden, F. C., and N. W. Pirie. 1952. Physiology of virus diseases. Ann. Rev. of Pl. Physiol. 3: 171-188.

TABLE 1. *Average numbers of local lesions/cm² of leaf area induced by tobacco mosaic virus on plants of Nicotiana glutinosa deficient in calcium and on those not deficient in calcium*

Exp. No.	Date	Lesions/cm² control	deficient
1	Feb. 1952	1.02(80)[a]	0.35(80)
2	Aug. 1952	0.12(59)	0.05(59)
3a	Feb. 1954	0.69(80)	0.30(80)
3b[b]	Feb. 1954	6.09(80)	1.96(80)

[a] The figures in parentheses represent the number of leaves used in the respective samples (half-leaves in experiment 3).
[b] Abrasive in inoculum.

paper reports the consistent and marked effects of calcium deficiency on reducing the number of local lesions on plants of *N. glutinosa* L. inoculated with the tobacco mosaic virus.

Essentially the same experimental procedures were followed for experiments on plants of *N. glutinosa* as were described in an earlier paper[3]. Calcium deficiency was induced by replacing calcium nitrate in the nutrient solution with sodium nitrate. Three days before inoculation, the growing points and the necrotic and distorted apical leaves characteristic of the deficiency were removed, as were the corresponding portions of the control plants. Four uppermost leaves on each plant were rubbed uniformly with a brush saturated with a suspension of tobacco mosaic virus, and rinsed with tap water. In one experiment, inoculations with and without Hyflo-Super-Cel in the inoculum were made on alternate half-leaves. Five days after inoculation the leaves were harvested, wet weighed, their areas determined, and local lesions counted (Table 1).

The differences in lesion counts between controls and deficients in all 3 experiments (Table 1) were significant at the 1 per cent level. If calcium deficiency affects susceptibility merely by reducing leaf area, no effect on lesions per unit area should be observed. However, such deficiency reduced lesions per unit area on the average by a factor of 2.7 and reduced leaf fresh weight by a smaller factor, 1.93. The results suggest, then, a specific reduction by calcium deficiency of infection of *N. glutinosa* by tobacco mosaic virus.

Tinsley[4] has shown that well-watered plants are more susceptible to infection by tobacco mosaic virus than are water-deficient plants. He reported that the cuticle was thinner in leaves of "wet" plants than in the "dry". Since susceptibility differences between the 2 groups of plants were partially abolished by the use of abrasive, he concluded that structural difference was concerned in part. The use of abrasive had no such effect in the work reported here (Table 1) which indicates that calcium deficiency affects intrinsic cell susceptibility to infection, rather than mechanical resistance to virus entry. — Department of Botany, Montana State University, Missoula, Montana.

[3] Chessin, M. 1951. The effects of nitrogen deficiency on the properties of tobacco-mosaic virus. Phytopathology 41: 235-237.

[4] Tinsley, T. W. 1951. The effects of variations in water supply on the susceptibility of plants to virus diseases. Ph.D. thesis, London University.

White Spot of Alfalfa as a Genetic Character[1]. Fred Reuel Jones[2]. From the first description of white spot of alfalfa in 1908[3] this conspicuous but minor disease of alfalfa appears to have been considered one to which all alfalfa plants presumably were equally susceptible, whatever the inciting agent the authors have indicated. The present note attempts to distinguish 2 kinds of white spot of alfalfa leaves that are genetically qualified. These 2 kinds of spots are not widely different in appearance, but they appear to be genetically qualified in different ways.

Both types of white spotting recorded here occurred in the greenhouse on plants that had been transplanted from the field in the late fall. The most common type of spotting appeared indistinguishable from that illustrated by Crabill,[4] who reproduced it experimentally by injuring crowns of transplanted plants, and by Richards[5] who reproduced it extensively by irrigating dry fields. Since the roots of the greenhouse plants had been severely pruned during transplanting from the field, and since the plants may have been watered inadequately at times in the greenhouse while they were making rapid growth just before blossoming, the time at which the spots first appeared, it was assumed that the same fundamental cause may have been operating as for Crabill and Richards. However, since white spot usually developed in growth from cuttings of spotted plants, but never in that from other plants, it was surmised that this white spot was not entirely due to accidental severe treatment, but that it might be genetically conditioned.

From the plants showing this type of white spot during the winter of 1943-44, 2 were crossed. One of these produced seed readily upon selfing. The 2 progenies thus obtained were put in the field in early spring, and in both a few white spots developed on lower leaves of about a third of the plants during the hot dry weather of late July. Such spotting was not found in any other populations set in the nursery that

[1] The writer wishes to acknowledge the support of the Agricultural Experiment Station, University of Wisconsin, and the Wisconsin Alumni Research Foundation, Madison, Wisconsin, in the performance of this work.

[2] Formerly Senior Pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture, in cooperation with the Wisconsin Agricultural Experiment Station.

[3] Stewart, F. C., G. T. French, and J. K. Wilson. 1908. Troubles of alfalfa in New York. N. Y. (Geneva) Agr. Exp. Sta. Bul. 305: 331-416.

[4] Crabill, C. H. 1916. Note on the white spot of alfalfa. Phytopathology 6: 91-93.

[5] Richards, B. L. 1929. White-spot of alfalfa and its relation to irrigation. Phytopathology 19: 125-141.

year, although it did occur occasionally in a few of the populations that had passed the previous winter in the field. One of the spotted parents crossed with a nonspotting plant in the greenhouse gave a population in which no white spots appeared in the nursery. Thus, these spotted plants when selfed and intercrossed appeared capable of producing some plants showing a little white spotting without being subjected to any extraordinary stress from root pruning. This evidence appears to indicate the genetic conditioning of this apparently common type of white spotting in alfalfa.

The second type of white spotting developed on a single plant in the greenhouse during the same winter. The spots were distinguished by being irregular in distribution on the leaf surface, by being irregular in outline, and by appearing only on the upper surfaces of leaves. Since this plant had great vigor and was irreplaceable in other work, crosses were made regardless of the white-spot defect. Seedlings from 3 crosses showed a similar spotting on the first unifoliate leaf of a few plants in the greenhouse, and the population obtained from selfing the spotted plants eventually showed spotting in about one-third of the plants. More or less distinct patterns in the arrangement of the spots was found on individuals, differing from stripes between veins to a ringspot similar to those frequently resulting from virus infections. The best distinguishing character common to all of the plants was the superficial appearance of most of the spots on the upper surface of the leaves. Thus, this type of white spot appeared distinct from the common type, not only in appearance, but also in genetic behavior. It appears, therefore, that the control of this so-called disease in new varieties may be to some extent in the hands of the plant breeder. Whether the white spotting and yellowing ascribed to potash deficiency is dependent in any degree upon inherited characters remains for further investigation.—Plant Pathology Department, University of Wisconsin, Madison, Wisconsin.

Phellem as a Diagnostic Symptom of Internal Cork of Sweet Potato.[1] A. S. WILLIAMS AND L. W. NIELSEN. The root symptoms of internal cork of sweet potato were described by Nusbaum[2] as dark brown to blackish necrotic lesions with phellem delimiting them from surrounding sound tissue. After a period of drying, the necrotic tissue becomes raised, changes in color from brownish-black to tan, and has a "corky" appearance. Anatomically the necrotic tissue is not phellem or cork, but the common name, internal cork, leaves this impression to one not familiar with the disease. Sweet potato necrosis would probably have been a more appropriate name for the disease.

TABLE 1. *Presence of phellem around internal cork disease lesions and other root lesions*

Type of lesion	Number lesions examined	Presence of phellem—per cent		
		Complete	Incomplete	Absent
Diseased roots stored at 80°F	100	12	83	5
Diseased roots stored at 55°F	100	45	43	12
Inoculated roots bedded in soil at 80°F	85	56	44[a]	
Root-knot nematode	115	47	53[a]	
Insect larval injury	26	100	0	

[a] Incomplete or absent.

Diagnosis of internal cork is difficult when the lesions are few and small in affected roots. For lesions approximately 2 mm or less in diameter, the presence of phellem has been used to diagnose the disease. Nusbaum[3] also described various foliage symptoms for the disease, but these symptoms are not always associated with the root symptoms[4, 5]. Root symptoms at present are the only reliable symptoms for making diagnosis. Since phellem around the necrotic area was first associated with the symptom complex, a study was made to determine the constancy of this tissue around small necrotic lesions. Hand sections were made of lesions that developed in diseased roots of the Porto Rico variety stored at approximately 80°F for 4 months and in similar cured roots stored at 55°F. Lesions from graft-inoculated healthy roots bedded in soil and held for 5 months in a greenhouse at 80-90°F and wounds made by root-knot nematodes and insect larvae also were examined. The root-knot and insect larval wounds were obtained from roots stored 4 months at approximately 80°F. The presence of phellem around these different lesions was determined by staining the sections with Sudan IV and examining with the microscope. The lesions were classified as having: 1) phellem well defined and completely surrounding the lesion, 2) phellem incompletely surrounding the lesion, or 3) phellem absent. The incomplete classification was based upon the development of necrosis in normal tissue outside a previously formed phellem or the failure of phellem to develop completely around a lesion. When the study was initiated the categories incomplete and absent phellem were not recognized and both were classified incomplete. The data are presented in Table 1.

[1] Contribution from Plant Pathology, North Carolina State College, Raleigh, North Carolina. Published with the approval of the Director as Paper No. 598 of the Journal Series.

[2] Nusbaum, C. J. 1946. Internal cork, a new disease of sweet potato of unidentified cause. Phytopathology 36: 18-23.

[3] Nusbaum, C. J. 1950. Internal cork of sweet potatoes. S. C. Agr. Exp. Sta. Bul. 381.

[4] Martin, W. J. 1950. Internal cork of sweet potatoes in Louisiana. (Abs.) Phytopathology 40: 789.

[5] Rankin, H. W. 1950. Studies of internal cork of sweet potatoes. (Abs.) Phytopathology 40: 790-791.

[6] Nielsen, L. W. 1952. Effect of temperature on the development of internal cork lesions in sweet potato roots. Phytopathology 42: 625-627.

The presence of phellem was influenced by storage temperature and by the type of lesion. A complete phellem was observed around only 12 per cent of internal cork lesions in roots stored at 80°F. In contrast, approximately 50 per cent of diseased roots stored at 55°F and of inoculated roots bedded in the greenhouse developed a complete phellem around lesions. Root-knot nematodes with egg masses also were surrounded by phellem in about half the cases, and the tunnels of all insect larvae had a complete phellem.

The size and number of lesions in diseased roots increases after harvest if the roots are stored at 70-80°F, but little change occurs when roots are stored at 55°F.[6] The data from this study also suggest that little change occurs in phellem development from the period of harvest (inoculated roots bedded in soil at 80°F) through several months of storage at 55°F. Largest differences in phellem development occurred when diseased roots were stored at 80°F. The higher percentage of incomplete phellem suggests a greater virus activity at this temperature. Necrosis apparently develops faster than the host responds to wall-off the area with phellem.

The variability of the presence of phellem around the necrotic areas of internal cork and its presence around nematodes and insect larval penetrations minimize its importance as a diagnostic symptom of the disease. Phellem is a host response delimiting a source of injury and develops around all wounds. On the basis of this study, small lesions having a well defined phellem in roots stored at 70-80°F are probably caused by some factor other than the internal cork virus.—North Carolina State College, Raleigh, North Carolina.

The Effect of Organic Acids on Tobacco Mosaic Virus Multiplication. FARID NOUR-ELDIN.[1] Several workers[2,3] have indicated that in animal tissues decreased respiration in the host may be accompanied by decreased multiplication of viruses. In animal tissues the Krebs cycle of organic acid metabolism, involved in host respiration, is well established. In higher plants, on the contrary, the metabolic pathways of organic acid metabolism remain uncertain. Some of the acids and enzyme systems in the Krebs cycle have been found in certain plants. It accordingly appeared desirable to determine whether organic acids affect virus formation.

Mature lateral leaves of Turkish tobacco plants were dusted with carborundum and inoculated with purified tobacco mosaic virus. Twenty-four hours after inoculation, 14-mm discs were punched from the inoculated leaves by means of a sharp cork borer. These discs were floated on solutions of organic acids in covered petri dishes. Control discs were floated on distilled water. Fifteen discs were placed in each petri dish, one disc from each leaf being placed in each dish. Ten ml of solution were used in each dish; the solutions and water were changed every 24 hours. The petri dishes were kept in a light chamber where electric lights provided an average light intensity of around 170 foot-candles. All organic acids were used at a concentration of 0.003 M; some of the acids were toxic to the host when more concentrated than 0.003 M. All solutions were adjusted to pH 5.6 with KOH solution.

The discs were floated 5-6 days on the solutions and were then washed in distilled water and frozen in closed vials. The virus assay procedure was essentially the heat-ultracentrifuge-spectrophotometer method described by Schlegel and Rawlins.[4]

The organic acids produced the following percentage increase in virus multiplication: citric acid, 20, 22, 19, 18, 21, 17, 23; alpha-ketoglutaric acid, 17, 14; succinic acid, 20, 24, 17; malic acid, 31, 27, 27. The results demonstrate that these organic acids caused a significant increase in tobacco mosaic virus multiplication under the experimental conditions used. Although the metabolic pathway or pathways by which organic acids increase virus multiplication have not been determined, it is evident that under the conditions of this experiment these acids have an important function in virus multiplication.—Department of Plant Pathology, University of California, Berkeley 4, California.

A Crown Rot of Sweet Corn Caused by Helminthosporium turcicum. R. S. COX AND E. A. WOLF. In the spring of 1954 a wilt disease was noted in several plots of Iowa No. 5125 inbred lines growing in the sweet corn disease nursery located at Pahokee, Florida. Later, several other inbred lines and hybrids became affected.

Symptoms were first evidenced by a sudden wilting of the leaves. This was followed rapidly by a marginal necrosis which progressed inward to the midribs, usually culminating in death of the entire plant within 2-5 days (Fig. 1).

Longitudinal sections of diseased plants revealed a darkened area at the crown (Fig. 1, right inset) which sometimes extended upward through the second node. Cross sections of the diseased area showed that the discoloration was restricted initially to the vascular bundles (Fig. 1, left inset). Roots appeared to be healthy except in advanced stages of the disease.

[1] Present address: Plant Pathology Section, Ministry of Agriculture, P. O. Orman, Giza, Egypt.

The author acknowledges the support of the Egyptian Government in providing a fellowship for support of this work.

Portion of a thesis submitted as partial fulfillment of the requirements for the Ph.D. degree, Plant Pathology Department, University of California, Berkeley.

[2] Ackermann, W. W. 1951. Concerning the relation of the Krebs cycle to virus propagation. Jour. Biol. Chem. 189: 421-428.

[3] Moulder, J. W. 1954. Biochemical aspects of the growth of feline pneumonitis virus in the chick embryo yolk sac. Bact. Rev. 18: 170-176.

[4] Schlegel, D. E., and T. E. Rawlins. 1953. A comparison of two methods of preparing tobacco mosaic virus for spectrophotometric assay. Phytopathology 43: 89-91.

FIG. 1. Crown and foliar symptoms of a plant killed as a result of crown infection with *Helminthosporium turcicum*. Left inset is an enlarged (2×) cross section of a portion of the diseased crown shown on the right. Note the discolored vascular bundles.

Isolations from diseased areas consistently yielded a species of *Helminthosporium*. All stages of the disease were readily reproduced by means of toothpick inoculations[1] of this fungus at the crown of potted plants.

Morphologically, the fungus appeared to be quite similar to *Helminthosporium turcicum* Pass., and lesions typical of northern corn leaf blight developed when the fungus was inoculated onto the leaves of potted plants. Plants crown-inoculated with known isolates of *H. turcicum* developed wilt and crown rot symptoms similar to those observed in the field. Isolates of *H. maydis* Nisikado & Miyake failed to produce such symptoms. Thus, it was concluded that the fungus in question was *H. turcicum*.

The disease described here appears to be similar in many respects to that reported by Stover[2] in 1922 which he attributed to an unnamed species of *Helminthosporium*. It differs, however, in that it was found on plants up to 2 months old rather than being confined to seedlings. Also, in the present work the roots were not usually affected, and diseased tissues were not noticeably shrunken.—Everglades Experiment Station, University of Florida, Belle Glade, Florida.

Infection of Peach with Apple Mosaic Virus. HUGH C. KIRKPATRICK.[1] Cochran[2] reported symptoms like those of apple mosaic in apple seedlings budded with peach buds containing ring spot virus. No symptoms on peach were recorded from inoculations with apple mosaic virus from 4 sources. Posnette[3] has reported negative results on peach and myrobalan seedlings inoculated by chip-budding with 4 strains of apple mosaic virus.

[1] Hildebrand, A. A. 1953. An elaboration of the toothpick method of inoculating plants. Canad. Jour. Agr. Sci. 33: 506-507.

[2] Stover, W. G. 1922. The relation of soil temperature to the development of the seedling blight of corn caused by Helminthosporium sp. (Abs.) Phytopathology 12: 30.

[1] Plant Pathologist, Horticultural Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Tree Fruit Experiment Station, Wenatchee, Washington.

[2] Cochran, L. C. 1950. Infection of apple and rose with ring-spot virus. (Abs.) Phytopathology 40: 964.

[3] Posnette, A. F. 1953. Virus transmissions between Prunus and Malus species. Rept. E. Malling Res. Sta. for 1952, p. 131.

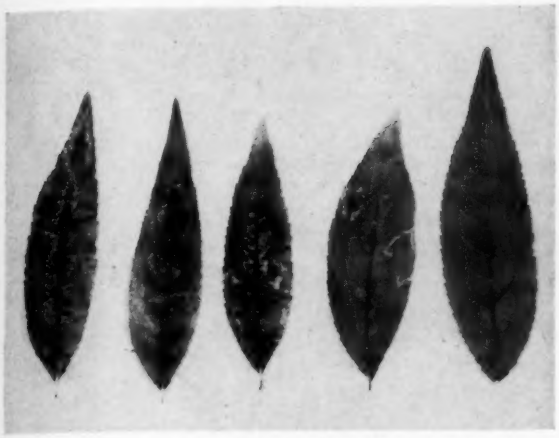


FIG. 1. Symptoms in Lovell peach seedling leaves following inoculation with apple mosaic virus.

In tests in the greenhouse during the fall of 1950, 3 Lovell peach seedlings were inoculated with apple mosaic virus by use of 2 buds and 1 leaf patch on each tree. No symptoms developed before the seedlings were placed in a cold room for the dormant period. In the fall of 1951, during the first flush of growth after a dormant period in a cold room, the 3 peach seedlings developed a faint line pattern which soon faded. In the summers of 1952 and 1953, these 3 seedlings again showed the faint line pattern in the first flush of growth after dormant periods in the cold room. (Fig. 1.) Following appearance of symptoms on the peach seedlings in 1952, 5 Golden Delicious apple seedlings were each budded with a single softwood bud from 1 of the Lovell peach trees. In addition, 3 Golden Delicious seedlings were inoculated with apple mosaic virus from the original source.

Apple mosaic symptoms developed in some of the apple seedlings in each group during the growing periods of 1952 and 1953. (Table 1.) The symptom patterns of apple mosaic on the Golden Delicious apple seedlings inoculated by means of buds from peach could not be distinguished from the patterns developing from infection with apple mosaic virus from the original source. There was no reaction on *Prunus serrulata* var. Shirofugen inoculated by budding from the check and peach-budded Golden Delicious seedlings showing apple mosaic symptoms, indicating that ring spot virus was not present in the apple.

These results indicate that peach is susceptible to apple mosaic virus, that it may carry this virus under greenhouse conditions, and that mild symptoms may develop in the first flush of growth. It seems possible that apple mosaic could exist and be overlooked in orchard peach trees.—Tree Fruit Experiment Station, Wenatchee, Washington.

TABLE 1.—*Production of apple mosaic symptoms in Golden Delicious apple seedlings budded with single softwood buds from Lovell peach seedlings that showed line patterns induced by apple mosaic virus*

Virus source	Date of inoculation	Symptoms[a] on indicated date		
		Aug. 1952	April 1953	May 1953
Peach 1	April, 1952	2/5	3/5	4/5
Peach 2	April, 1952	4/5	4/5	5/5
Peach 3	April, 1952	4/5	4/5	4/5
Apple[b]	July, 1952	0/3	1/3	2/3

[a] Denominator represents number of test trees and numerator number showing symptoms.

[b] Apple mosaic virus from original source used as check.

Asexual Reproduction in Cephalosporium gregatum[1]. RICHARD I. HAMILTON AND MICHAEL G. BOOSALIS[2]. In studies on the brown stem rot of soybean incited by *Cephalosporium gregatum* Allington & Chamberlain[3], difficulty was experienced in obtaining consistent sporulation of the organism on artificial media. Moreover, circumstantial evidence was obtained of the occurrence of a budding type of conidial germination. A process analagous to budding has been reported for *Cephalosporium pamelli* by Buchanan[4], in which allantoid septate conidia often were seen to produce slender pegs bearing replicas of the parent conidium. Successive "buds" remained attached one to another by these pegs.

Accordingly, investigations were made to determine some of the factors influencing sporulation, and also to elucidate the manner in which conidia of *C. gregatum* germinate. This paper presents the results of these studies.

The 2 isolates used in the study on factors influencing sporulation were obtained from D. W. Chamberlain of the U. S. Regional Soybean Laboratory, Urbana, Illinois and M. F. Kernkamp, Division of Plant Pathology, University of Minnesota, St. Paul, Minnesota. The isolates were cultured in liquid shake-cultures or on solid media of potato or soybean decoction. The effects on sporulation of temperature, concentration of hydrogen ions, presence or absence of dextrose, and successive transfers to fresh media were studied.

Three strains from the Illinois isolate and 1 from the American Type Culture Collection were used for conidial germination studies. The isolates were cultured on 2 per cent Bacto water agar at pH 6.5 on sterile microscope slides at 21°C. Microscopic exam-

[1] Contribution from the Department of Plant Pathology, Nebraska Agricultural Experiment Station, Lincoln, Nebraska. Published with the approval of the Director as Paper No. 681, Journal Series of the Nebraska Agricultural Experiment Station.

[2] Research Assistant and Assistant Plant Pathologist, respectively.

[3] Allington, W. B., and D. W. Chamberlain. 1948. Brown stem rot of soybean. Phytopathology 38: 793-802.

[4] Buchanan, R. E. 1911. Morphology of the genus Cephalosporium with description of a new species and a variety. Mycologia 3: 170-177.

inations were made by placing a drop of sterile immersion oil directly upon a sporulating colony and then examining it with an oil immersion lens at ×970 magnification. Line drawings, made with the use of a camera lucida, afforded a means of recording with considerable clarity the daily process of conidial production and germination.

Dilution plates from shake cultures of *C. gregatum* yielded both sparse-sporulating and nonsporulating colonies. Presley and Allington[5] first reported difficulty in obtaining conidial production except on certain media. Preliminary attempts by the authors to influence sporulation likewise generally were unsuccessful.

Temperatures between 14.5°C and 21.0°C were found to promote sporulation, but fruiting was not recorded at temperatures above 29.0°C. It was not possible to induce spore production in the nonsporulating type at any temperature. The hydrogen ion concentration in the medium was found to influence fruiting, with good sporulation occurring between pH 5.0 and 6.0. No effect of pH was observed with the nonsporulating type. Moreover, successive transferring of mycelium to fresh potato dextrose agar every 2 weeks for over 1 year did not induce sporulation in the nonsporulating type. Soybean stem agar lacking dextrose, as described by Allington and Chamberlain[3], was found to induce sporulation in the nonsporulating type, but when pieces of mycelium were transferred back to potato dextrose agar, sporulation was not observed. Sporulation of the sporulating type was not affected by the presence or absence of dextrose. Single spore isolates of both types, when transferred to media containing dextrose, behaved in the same manner as did masses of inoculum.

In the study on conidial germination, microscopic observations of the 4 isolates used demonstrated that the common method of asexual reproduction is by the production of hyaline, nonseptate, ovoid to elliptical conidia, as reported by Allington and Chamberlain[3]. The majority of these conidia remained dormant until conditions were conducive for the typical process of germination by germ tube. However, in many instances, conidia elongated within 24-36 hours after production and formed a definite septum which traversed the conidium approximately midway between its linear

[5] Presley, John T., and W. B. Allington. 1947. Brown stem rot of soybean caused by a Cephalosporium. Phytopathology 37: 681-682.

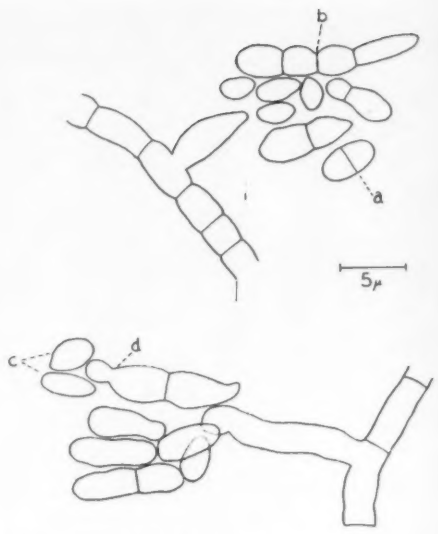


FIG. 1. Camera lucida drawings of *C. gregatum* on water agar. a) conidium with septum midway between linear extremes; b) elongated conidium which has laid down additional septations; c) budded conidia lying adjacent to parent conidium; d) parent conidium showing bud-like projection which later was separated. ×970.

extremes (Fig. 1, a). The septate conidia frequently continued to elongate to several times their original length, and additional septations were often formed (Fig. 1, b). An abstriction of 1 end of a septate conidium gave rise to a bud-like projection which, in many cases, became separated from the parent conidium (Fig. 1, c, d). These buds in all cases were hyaline, ovoid to elliptical, and nonseptate.

The production of buds was observed to take place only by the elongated and septate conidia, and it has yet to be demonstrated on media other than water agar. Budding was successive in several instances; the maximum number of buds observed being produced by a parent conidium was 3. However, as many as 10 bud-like conidia were seen around the tips of many elongated and septate conidia. Although the actual demonstration of budding was seen to occur under the abnormal environment created by flooding the colony with immersion oil, the presence of many bud-like conidia adjacent to the tips of elongated conidia in colonies not covered with oil suggested that this phenomenon may occur frequently.—Department of Plant Pathology, Nebraska Agricultural Experiment Station, Lincoln, Nebraska.

For **MODERN** Plant Nutrition

Tennessee Corporation, the leading supplier of trace minerals to the nation's growers, announces two additional products for improved plant nutrition.

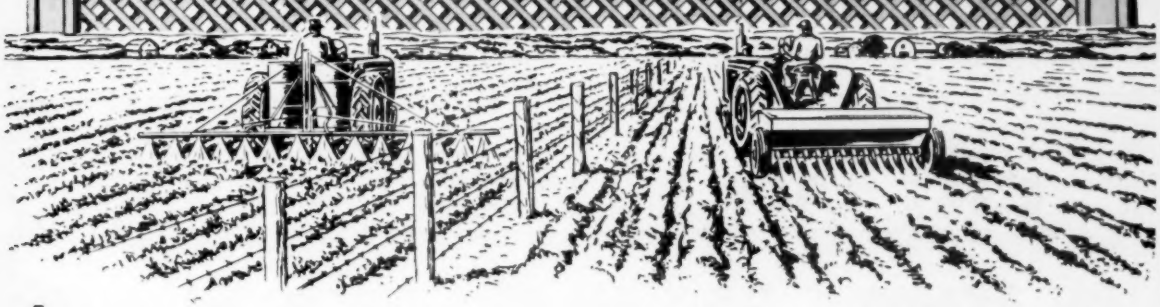
NU-MANESE

(Manganous Oxide)

For soil application, for use in mixed fertilizer or for direct spray or dust application.

NU-IRON

For correction of Chlorosis resulting from iron deficiencies by spray or dust application to the plant.

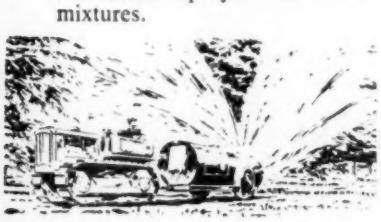


The above supplements our widely accepted agricultural chemicals.

NU-M	**NU-Z**
TRI-BASIC COPPER SULFATE	**COP-O-ZINK**
ES-MIN-EL Spray or Dust	**ES-MIN-EL** For Soil Application

DUST MIXERS:

Tennessee's **NU-M**, **NU-Z**, **NU-IRON** and **TRI-BASIC** Copper Sulphate are especially suited to be used in preparing nutritional sprays and dust mixtures.



TENNESSEE CORPORATION

617-629 Grant Building, Atlanta, Ga.

Pathologist Frank A. Haasis, Professor of Plant Pathology, North Carolina State College, will be Co-Director of the Agricultural Experiment Station at Tingo, Maria, Peru, for a two-year period.

A Working Party on Seed-Borne Diseases, convened by the European Plant Protection Organization (EPPO) in collaboration with the International Seed Testing Association (ISTA), was held at Paris, 2-3 July 1954. Its participants included several members of the ISTA Committee on Seed-borne Diseases and Pests, and a representative of FAO.

Plant Pathologist W. J. Hooker formerly Associate Professor of Botany and Plant Pathology, Iowa State College has replaced the late Dr. J. H. Muncie as potato pathologist at Michigan State College, East Lansing.

Members of the Northeastern Division visited the fruit and vegetable markets and quarantine inspection stations of the Port of New York by chartered bus on April 5. The tour started at 4:00 a.m. with an inspection of the fruit and vegetable markets at Washington Street and the Pennsylvania and Erie Railroad piers. Dr. B. A. Friedman explained the operations of the markets. The group then returned to the headquarters at the Hotel New Yorker for breakfast. During the day, New York piers, the International Airport and the Federal Plant Inspection House at Hoboken, New Jersey, were visited. The facilities for inspection and fumigation of plant materials, as well as actual inspection work, were observed. Messrs. George G. Becker, R. D. Whitlock, R. G. Oakley and Emil Kostal explained the work of the plant quarantine service. The tour concluded at the Federal Building where the members observed an excellent exhibit of diseases and insect pests. A dinner was served by members of the quarantine staff followed by a question and answer period. Arrangements for the tour were made by E. G. Rex.

Pathologist Kenneth H. Garren formerly of the Alabama Polytechnic Institute and later Agricultural Research Advisor to the Ministry of Agriculture of El Salvador has returned to the United States and accepted an appointment as Plant Pathologist with the U.S.D.A. at the Tidewater Research Station, Virginia Agricultural Experiment Station, Holland, Virginia.

Deaths: Lucia McCollough, retired U.S.D.A. plant pathologist passed away recently following a period of failing health. Miss McCollough joined the Department in 1905 working at one time with Dr. Erwin F. Smith. She retired in 1941.

TRIARCH PRODUCTS

FINE MICROSCOPE SLIDES FOR DISCRIMINATING BIOLOGISTS

As we prepare and sell only microscope slides, we are able to give you better quality, better service and better prices than you can get elsewhere.

Remember, TRIARCH means ACCURACY, DEPENDABILITY, SERVICE

Write for our illustrated catalog No. 10

GEO. H. CONANT, RIPON, WISCONSIN